A Phase 2, Multicenter, Open-label Study to Evaluate the Pharmacokinetics and Safety of RLYB212 in Pregnant Women at Higher Risk for HPA-1a Alloimmunization.

Unique Protocol ID: IPA2202

NCT Number: NCT06435845

EudraCT Number: 2024-512651-20-00

Date of Protocol: 14 October 2024

TITLE PAGE

Protocol Title		A Phase 2, Multicenter, Open-label Study to Evaluate the Pharmacokinetics and Safety of RLYB212 in Pregnant Women at Higher Risk for HPA-1a Alloimmunization	
Protocol Number		IPA2202	
Compound		RLYB212	
Brief Title		Phase 2 Study on the Pharmacokinetics and Safety of RLYB212 in Pregnant Women at Higher Risk for HPA-1a Alloimmunization	
Study Phase		2	
Sponsor	**Name**	Rallybio IPA, LLC	
	Legal Registered Address	[redacted] New Haven, CT 06510	
	EU Legal Representative	FGK Representative Services BV	
Regulatory Agency Identifier Number(s)		IND: Not available EU CT: 2024-512651-20 NCT: NCT06435845 WHO: Not available EUDRANET: Not available Other: Not applicable	
Protocol Date		**Document Version**	**Date**
		Version 1.0	11 March 2024
		Version 1.1	23 August 2024
		Version 2.0	04 September 2024
		Version 3.0	25 September 2024
		Version 4.0	14 October 2024

CONFIDENTIALITY NOTICE

The information contained in this protocol and all other information relevant to RLYB212 are the confidential and proprietary information of Rallybio IPA, LLC and, except as may be required by federal, state, or local laws or regulation, may not be disclosed to others without the prior written permission of Rallybio IPA, LLC.

SPONSOR SIGNATORY

IPA2202: A Phase 2, Multicenter, Open-label Study to Evaluate the Pharmacokinetics and Safety of RLYB212 in Pregnant Women at Higher Risk for HPA-1a Alloimmunization.

I, the undersigned, have approved version 4.0 of the clinical trial protocol with the date of 14 October 2024.

Name and Title	Signature and Date
[redacted] Chief Medical Officer Rallybio IPA, LLC	[redacted]

INVESTIGATOR AGREEMENT

IPA2202: A Phase 2, Multicenter, Open-label Study to Evaluate the Pharmacokinetics and Safety of RLYB212 in Pregnant Women at Higher Risk for HPA-1a Alloimmunization.

I have read the protocol, including all appendices, and I agree that it contains all the necessary information for me and my staff to conduct this study as described. I will conduct this study as outlined herein, in compliance with current Good Clinical Practice (GCP) standards as defined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for GCP, all applicable national, state, and local laws and regulations, and the applicable Institutional Review Board/Independent Ethics Committee (IRB/IEC) and other institutional requirements.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by Rallybio or specified designees. I will discuss the material with them to ensure that they are fully informed about RLYB212, understand this study, and are able to comply.

_____ _____

Principal Investigator Name (printed) Signature

_____

Date

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A Phase 2, Multicenter, Open-label Study to Evaluate the Pharmacokinetics and Safety of RLYB212 in Pregnant Women at Higher Risk for HPA-1a Alloimmunization

Short Title: Phase 2 Study on the Pharmacokinetics and Safety of RLYB212 in Pregnant Women at Higher Risk for HPA-1a Alloimmunization

Rationale: RLYB212 is a human monoclonal anti-human platelet antigen (HPA)-1a immunoglobulin G antibody designed to selectively bind to HPA-1a positive fetal derived cells or cell fragments present in the maternal circulation. The therapeutic goal of RLYB212 is to drive the rapid and complete elimination of HPA-1a positive fetal antigen from the maternal circulation in HPA-1b/b pregnant women and prevent maternal HPA-1a alloimmunization.

A prophylactic dose regimen consisting of a single 0.12 mg subcutaneous (SC) loading dose of RLYB212 no later than gestational week (GW) 16 followed by 0.06 mg SC every 4 weeks (Q4W) through parturition is proposed for HPA-1b/b pregnant women at risk for HPA-1a alloimmunization. This dose regimen is the result of exposure simulations in pregnant women using a clinical pharmacology model based on pharmacokinetic (PK) and pharmacodynamic (PD) single and multiple dose data from two clinical studies with RLYB212 in HPA-1b/b volunteers, a meta-analysis of monoclonal antibody PK parameters, allometric scaling to account for demographic differences between study populations (Phase 1/non-pregnant and pregnant), and the dynamic physiologic changes that occur during gestation. The dose regimen is intended to achieve near steady state RLYB212 concentrations after the first dose and for steady state concentrations to be maintained within a target range of ~3 ng/mL to ~10 ng/mL for the duration of the pregnancy.

The target lower boundary is supported by clinical and preclinical data demonstrating that concentrations of RLYB212 sufficient to bind ~10% of HPA-1a positive receptors in an antigen challenge model are effective to drive rapid elimination of circulating HPA-1a positive antigen positive cells. The target upper boundary of ~10 ng/mL is supported by a comprehensive toxicology program that demonstrated RLYB212 to have no observed adverse effect level (NOAEL) margins of up to 242-fold greater than 10 ng/mL in antigen negative dams bearing heterozygous antigen positive offspring (representative of HPA-1b/b women bearing an HPA-1a positive fetus and at risk of alloimmunization) and projected exposures well over 10-fold greater than 10 ng/mL in antigen positive fetuses and neonates (representative of HPA-1a/b fetuses and neonates). The target upper boundary is further informed by an extensive review of available literature on clinical assessments of HPA-1a alloimmunization and fetal and neonatal alloimmune thrombocytopenia (FNAIT), where a maternal HPA-1a alloantibody level of 3 IU/mL has been reported as the threshold for the occurrence of anti-HPA-1a antibody-mediated fetal/neonatal thrombocytopenia. Using the most conservative estimates for binding equivalence in IU for RLYB212, the steady state upper boundary exposure target accounts for an additional margin of safety by maintaining RLYB212 concentrations at or below ~0.5 IU/mL.

The purpose of this Phase 2 study is to assess the PK and safety of RLYB212 at the proposed therapeutic dose regimen in HPA-1b/b pregnant women at higher risk for HPA-1a alloimmunization and FNAIT. Following confirmation of the prophylactic RLYB212 dose regimen, it is planned to evaluate the safety and efficacy of RLYB212 in a Phase 3 registration trial in pregnant women at higher risk for the occurrence of HPA-1a alloimmunization and FNAIT.

Objectives and Endpoints (Interventional/Study IPA2202)

Objective	Endpoint
Primary	
• To evaluate the PK profile of RLYB212 during pregnancy following repeat SC administration	• Maternal PK parameters, eg, ○ half-life of RLYB212 ($t_{½}$) ○ maximum RLYB212 concentration (C_{max}) ○ time to maximum RLYB212 concentration (t_{max}) ○ apparent clearance (CL/F) of RLYB212 ○ apparent volume of distribution (Vd) ○ area under the RLYB212 concentration versus time curve (AUC)
• To assess maternal and fetal safety of RLYB212 during pregnancy	• Type, seriousness, and incidence of AEs • Physical examination findings • Vital signs • Maternal clinical laboratory values • ECG • Obstetric/fetal doppler ultrasound
Secondary	
• To evaluate RLYB212 exposure in the neonate at delivery	• Concentration of RLYB212 in cord blood
• To assess the safety of RLYB212 in the HPA-1a positive neonate	• Type, seriousness, and incidence of AEs including fetal/neonatal AEs • Physical examination findings, including APGAR scores • Vital signs
• To assess the immunogenicity of RLYB212	• Development of ADAs
• To assess pregnancy and neonatal outcomes following antenatal RLYB212 administration	• Number of spontaneous abortions • Number of elective abortions • Number of stillbirths • Number of premature births • Number of full term births (≥ 37 completed weeks of gestation) • Overall health and development of infants at 4-6 weeks and 12 months of age
• To assess the occurrence of neonatal thrombocytopenia following antenatal RLYB212 administration	• Neonatal thrombocytopenia[a] and severe neonatal thrombocytopenia
• To assess the occurrence of HPA-1a alloimmunization	• Presence of maternal anti-HPA-1a alloantibodies at Week 10 post pregnancy[b]

ADA = anti-drug antibody; AE = adverse event; APGAR = Appearance, Pulse, Grimace, Activity, and Respiration; ECG = electrocardiogram; HPA-1a = human platelet antigen 1a; PK = pharmacokinetic(s); SC = subcutaneous.

[a] Evaluation of neonatal thrombocytopenia will be based on the umbilical cord sample taken at parturition.

[b] For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

A non-interventional (observational) sub-study (IPA2202A) is planned based on the phased approach to participant enrollment that results from the sequential cohort design. Because of the rarity of the potential occurrence of HPA-1a alloimmunization, the non-interventional (observational) sub-study will serve to augment an ongoing FNAIT Natural History Study (NCT05345561), ie, generating natural history data on the occurrence of HPA-1a alloimmunization and pregnancy/neonatal outcomes in women at higher FNAIT risk. Descriptions of the non-interventional (observational) sub-study design are presented separately across this protocol.

Objectives and Endpoints (Non-interventional [Observational]/Study IPA2202A)

Objective	Endpoint
• To inform the frequency of HPA-1a alloimmunization among pregnant women identified at higher FNAIT risk	• Occurrence of anti-HPA-1a maternal alloimmunization at Week 10 postpartum[a]
• To inform the frequency of pregnancy outcomes among pregnant women identified at higher FNAIT risk	• Rate of spontaneous abortion, defined as non-deliberate fetal death which occurs prior to 19 weeks of gestation • Rate of elective abortion, defined as deliberate termination of pregnancy at any time in gestation • Rate of stillbirth, defined as non-deliberate fetal death anytime in gestation on or after 19 weeks of gestation • Rate of premature birth, defined as live birth prior to 37 completed weeks of gestation • Rate of full term birth (≥ 37 completed weeks of gestation)
• To inform the frequency, where data are available, of neonatal thrombocytopenia in infants born to women who have alloimmunized, as determined by detectable anti-HPA-1a antibody at Week 10 postpartum[a]	• Neonatal thrombocytopenia and severe neonatal thrombocytopenia, as determined by a platelet count $< 150 \times 10^9$/L and $< 50 \times 10^9$/L, respectively, where data are available

[a] For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

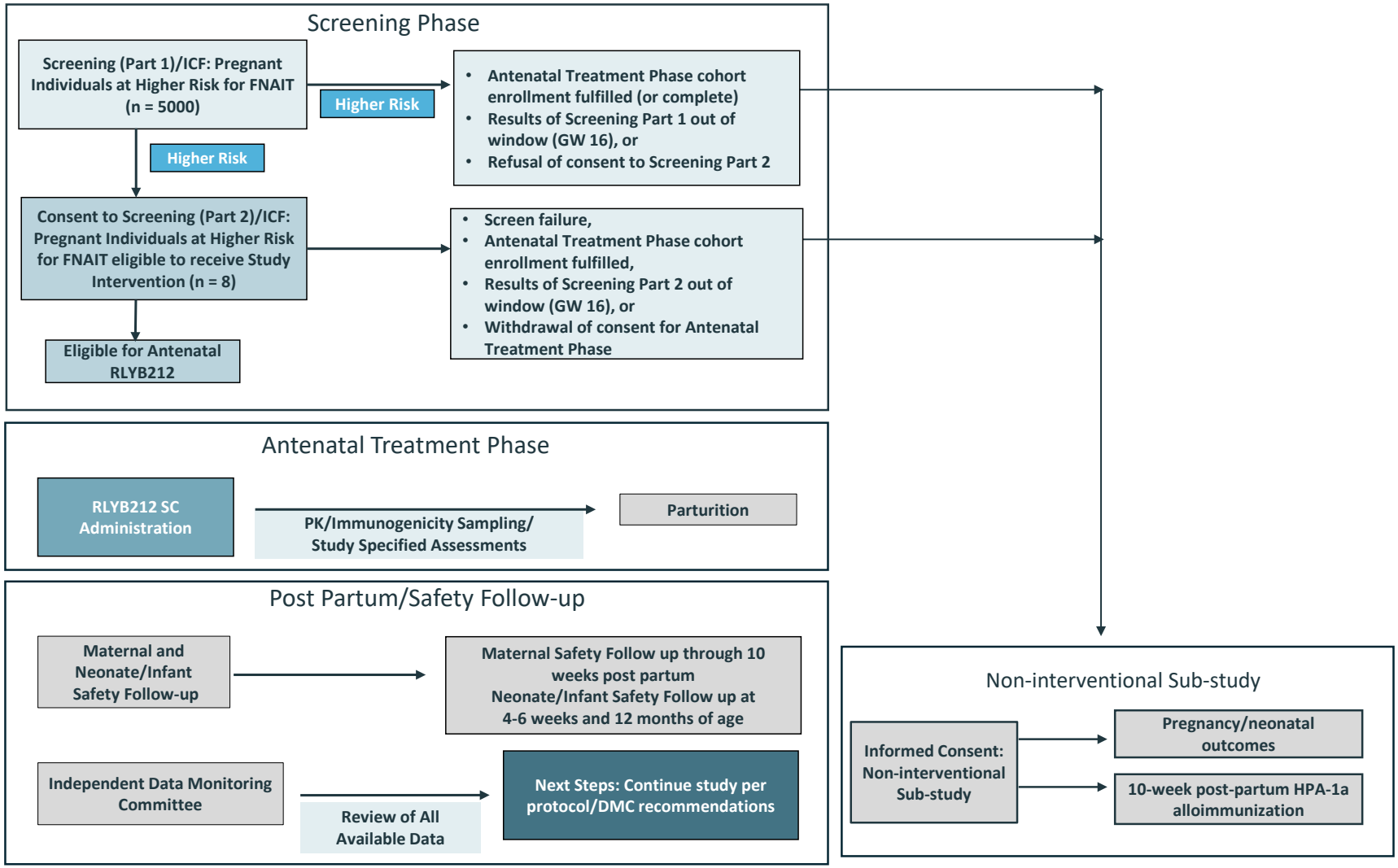
Overall Design

This study is a single-arm, open-label, multicenter study of RLYB212 in HPA-1b/b pregnant participants at higher risk for the occurrence of HPA-1a alloimmunization and FNAIT. A laboratory testing paradigm will be applied at screening to identify women at higher risk for HPA-1a alloimmunization. Study IPA2202 is comprised of three phases: a two-part screening phase, an antenatal treatment phase, and a postpartum follow-up phase (Figure 1-1). Study duration for each participant is anticipated to be ~44 weeks, inclusive of the screening visits through the Week 10 postpartum visit. For each child born during the study, the duration is anticipated to be ~12 months.

For the non-interventional (observational) sub-study (IPA2202A), the same two-part screening phase for the intervention, as applicable, is followed by a postpartum/safety follow-up phase. The sub-study duration is anticipated to be ~44 weeks, inclusive of the screening visits through the Week 10 postpartum visit.

The overall design of the Phase 2 study, including the non-interventional (observational) sub-study, is presented in Figure 1-1.

Figure 1-1 Overall Study Design (Interventional and Non-interventional [Observational])



FNAIT = fetal and neonatal alloimmune thrombocytopenia; ICF = informed consent form; PK = pharmacokinetic; SC = subcutaneous.
For Screening (Part 2)/ICF: Enrollment planned for 8 eligible participants for whom the pregnancy results in a live birth and who are evaluable for PK and safety assessments.

Screening Phase

In participants presenting at GW 6 or after who provide informed consent, blood samples will be collected at the Screening Part 1 visit to assess for higher risk for the occurrence of HPA-1a alloimmunization during their pregnancy. The first test will be a test for maternal HPA-1 genotype. In participants identified as HPA-1b/b (ie, HPA-1a negative), subsequent tests will be performed to confirm the participant has an HLA-DRB3*01:01 positive genotype, is anti-HPA-1a alloantibody negative, and is carrying an HPA-1a/b (ie, HPA-1a positive) fetus.

Participants who meet all eligibility criteria in Screening Part 1 (ie, HPA-1b/b and HLA-DRB3*01:01 positive genotype; anti-HPA-1a alloantibody negative; fetal HPA-1a/b genotype) and who provide informed consent for Screening Part 2 (Visit 1/schedule of activities [SoA], Table 1-3) will be assessed to determine if they meet all eligibility criteria to enter the antenatal treatment phase of the study and to receive RLYB212 in accordance with protocol dose regimen.

Antenatal Treatment Phase

For participants who meet all the eligibility criteria at Screening Part 2 (Visit 2/Table 1-3), dosing with RLYB212 will be initiated at Visit 3, which must occur no later than GW 16. If the fetal HPA-1 genotype is pending and will not be available by the planned visit at ~GW 16, the subject will be dosed with the initial dose of RLYB212 pending the result (of the replicate tests), provided all other Screening Part 2 criteria are met. A second dose of RLYB212 will not be given if definitive fetal HPA-1a results are not available by Visit 4 (~GW 20). If the fetal HPA-1a genotype data are only available after the initial dose of RLYB212 is given and the fetus is HPA-1b/b, ie, not at risk for HPA-1a alloimmunization, the participant will be discharged from the study to receive normal prenatal care and follow-up by their physician. In this case, the participant would have been determined not to be at risk for development of HPA-1a alloimmunization and FNAIT.

Dosing will be continued at the protocol-specified dose regimen during the pregnancy with the final dose of RLYB212 administered within 24 hours (±24 hours) of parturition.

Postpartum/Safety Follow-up

Upon completion of the antenatal treatment phase, participants will be monitored through 10 weeks postpartum. Safety follow-up for the neonate/infant will be at 4-6 weeks and 12 months following parturition. For pregnancies that do not result in a live birth, monitoring will be for 10 weeks from the date of the pregnancy terminating event.

Statistical Considerations

No statistical hypothesis testing will be performed in this study. No formal interim analyses will be conducted. Preliminary data may be used for summary purposes and for communication with health authorities.

Number of Participants

It is anticipated that approximately 5,000 participants will be screened to enroll 8 eligible participants for whom the pregnancy results in a live birth and who are evaluable for PK and safety assessments. Up to 4 participants may be replaced if they were assigned to study drug but did not receive the study drug. Participants may be replaced for reasons other than clinical safety, including insufficient PK sample collection to characterize pharmacokinetics.

Eligibility Criteria

Eligibility for this study will be established using a two-part screening procedure. Screening Part 1 will identify those participants who are at higher risk for the occurrence of maternal HPA-1a alloimmunization and FNAIT in their pregnancy. Women in their first or subsequent pregnancy may be included in the study. If a participant meets eligibility criteria in Screening Part 1, they may proceed to Screening Part 2 to determine their eligibility for enrollment and subsequent treatment with RLYB212. If a participant is eligible at Screening Part 2 for the antenatal treatment phase, but the cohort has been fully enrolled, the participant will be requested to consent for enrollment in the non-interventional (observational) sub-study (Table 1-1).

Inclusion Criteria to be Confirmed at Screening Part 1

1. Written/signed informed consent (for Screening Part 1)
2. 18-45 years of age
3. Pregnant women who present at GW 6 or after and confirmed to be:
    a. HPA-1b/b (HPA-1a negative)
    b. HLA-DRB3*01:01 positive
    c. Anti-HPA-1a alloantibody negative
    d. Carrying an HPA-1a/b (HPA-1a positive) fetus

Exclusion Criteria to be Confirmed at Screening Part 1

1. Prior history of HPA-1a-related FNAIT
2. Prior history of HPA-1a alloimmunization
3. Prior history of platelet transfusion or other blood transfusions
4. Body mass index (BMI) ≥ 35 kg/m^2

Eligibility Criteria for Study Intervention

Participants who meet all eligibility criteria in Screening Part 1 (ie, HPA-1b/b and HLA-DRB3*01:01 positive genotype; anti-HPA-1a alloantibody negative; fetal HPA-1a/b

genotype) and who provide informed consent for Screening Part 2, will be assessed for eligibility to enter the study's antenatal treatment phase and to receive treatment with RLYB212 provided all inclusion and no exclusion criteria are met.

Inclusion Criteria for Study Drug Administration to be Confirmed at Screening Part 2

1. Written/signed informed consent (for Screening Part 2)

2. 18-45 years of age

3. Pregnancy no later than GW 16 at the time of first study drug administration as determined by the principal investigator or qualified staff

Exclusion Criteria for Study Drug Administration to be Confirmed at Screening Part 2

Participants are excluded from the study if any of the following criteria apply:

1. Multiple pregnancy (> 1 fetus)

2. Known sensitivity and/or immediate hypersensitivity to any components of RLYB212 or its formulation

3. History of hypersensitivity to protein therapeutics

4. Any participant with a history of past or current intravenous immunoglobulin (IVIG) use for an underlying disease (eg, autoimmune disease)

5. Requires ongoing systemic corticosteroid or systemic immunosuppressive treatment for any reason

6. History of pre-eclampsia in a previous pregnancy

7. Participants with an in vitro fertilized pregnancy or serving as pregnancy surrogates

8. Current (or within the last 90 days) participation in another clinical study and have received an investigational drug and device within the last 90 days

9. Participants known or suspected of not being able to comply with this study protocol

10. History of any connective tissue or autoimmune or autoinflammatory disease, especially autoimmune thrombocytopenia

11. Any co-morbid medical or obstetric condition(s), laboratory abnormality, concomitant treatment, or other reason that, in the investigator's opinion, could adversely affect the safety of the participant and/or fetus, impair the assessment of study results, or preclude compliance with the study.

Non-interventional (Observational) Sub-study (IPA2202A) Eligibility and Population

It is anticipated that a limited number of participants will be enrolled in the non-interventional (observational) sub-study. Criteria for enrollment in the sub-study, with reference to the two-part Screening phase, include the following:

Table 1-1 Anticipated Eligibility Criteria for Sub-study

Screening Part 1	Screening Part 2
In the Antenatal Treatment Phase, the enrollment for the current sentinel or sequenced cohort has been fulfilled	
Results of Screening are only available after the first study drug administration window (by GW 16) has passed	
Refusal of consent to Screen Part 2	Screen Failure
---	Withdrawal of consent for Antenatal Treatment Phase

Intervention Groups and Duration

All interventional participants will receive SC RLYB212 administered using a vial and syringe.

RLYB212 treatment will be initiated at the dosing visit (Visit 3) with an initial SC dose of 0.12 mg no later than GW 16, followed by SC doses of 0.06 mg Q4W and with a final 0.06 mg dose administered within 24 hours (±24 hours) of parturition.

A key goal of the study is to define the RLYB212 dose regimen that achieves the pre-determined target exposure range through parturition. Modification of the RLYB212 dose regimen may therefore be necessary, to ensure desired steady state concentrations are maintained through pregnancy and parturition between a target lower bound of ~3 ng/mL and a target upper bound of ~10 ng/mL.

Modification of RLYB212 dose/dose frequency may be made within a cohort or between cohorts, based on available PK data from participant(s) with a completed pregnancy. Dose modifications are not anticipated during the pregnancy of an individual participant.

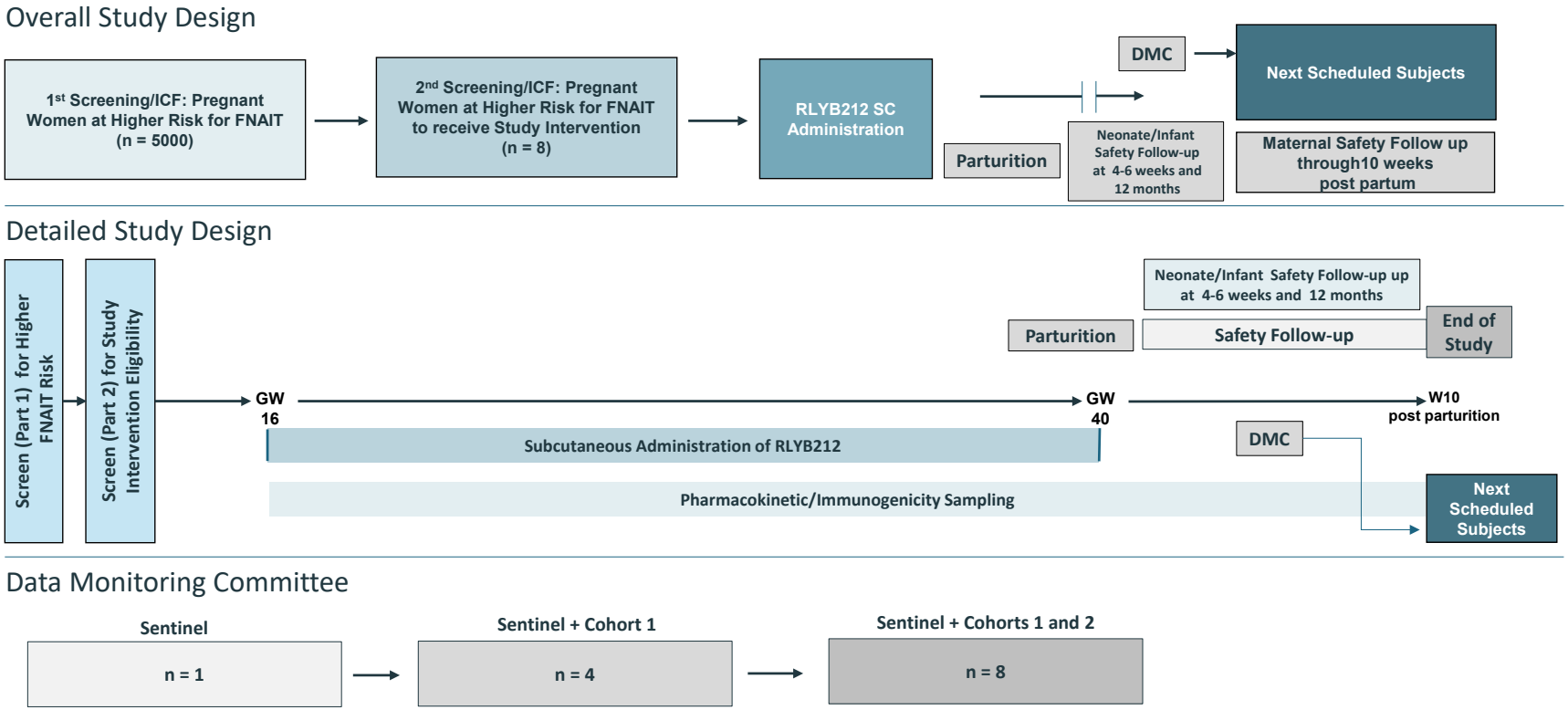
Data Monitoring Committee

An independent data monitoring committee (DMC) will monitor safety outcomes and study conduct and will provide recommendations for consideration by the Sponsor regarding the continuation of the study, dosing of further participants in the study, or stopping the study for all participants or subgroups of participants in the study. Safety data will include, but not be limited to, those outcomes listed as primary and secondary endpoints.

The Sponsor will propose a detailed mandate to the DMC and review this with the DMC prior to the initiation of screening. A detailed DMC charter and operating rules will be prepared and signed by DMC members before study start.

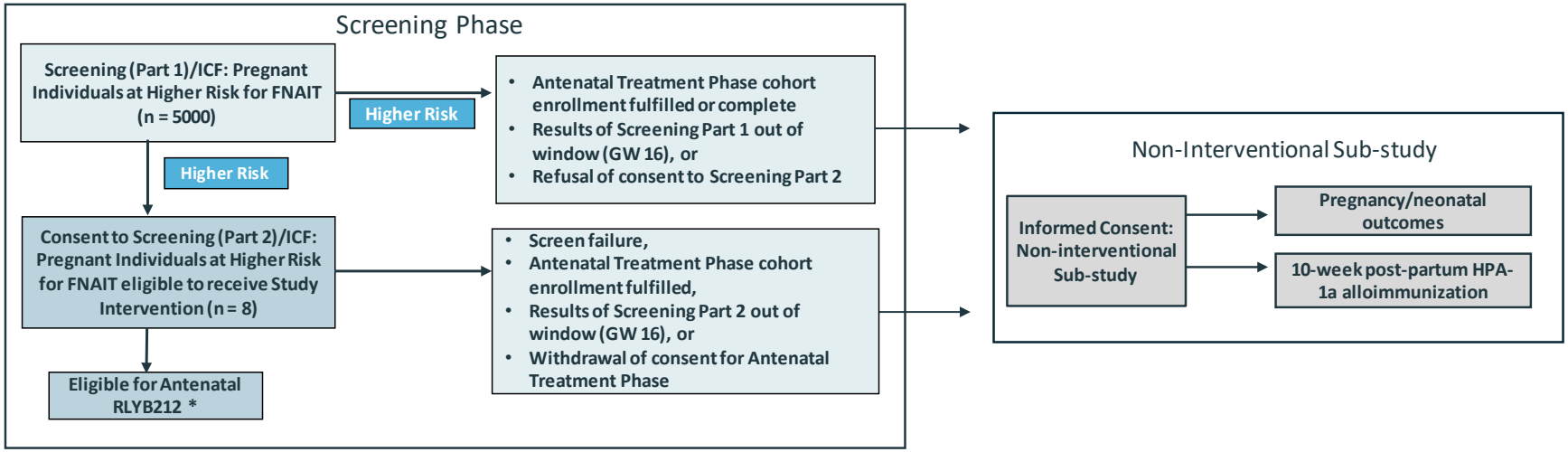
1.2 Schema

1.2.1 Interventional Study (IPA2202)



DMC = data monitoring committee; FNAIT = fetal and neonatal alloimmune thrombocytopenia; GW = gestational week; ICF = informed consent form; n (for DMC section) = total number of participants receiving the intervention; SC = subcutaneous; W = week.
For Screening (Part 2)/ICF: Enrollment planned for 8 eligible participants for whom the pregnancy results in a live birth and who are evaluable for PK and safety assessments.

1.2.2 Non-interventional (Observational) Sub-study (IPA2202A)



FNAIT = fetal and neonatal alloimmune thrombocytopenia; GW = gestational week; HPA = human platelet antigen; ICF = informed consent form; W = week.
* Refer to Section 1.2.1.

1.3 Schedule of Activities (SoA)

Quick Link	
Table 1-2	Schedule of Activities: Screening Part 1 and Screening Part 2
Table 1-3	Schedule of Activities: Eligibility for Study Intervention, Antenatal Treatment, and Postnatal Follow-up
Table 1-4	Schedule of Activities: Subjects Eligible for Non-interventional (Observational) Sub-study and Follow-up

Table 1-2 Schedule of Activities: Screening Part 1 and Screening Part 2

Assessments	Notes
Screening Part 1	
Informed consent (SCR)	Consent for blood samples to be collected to determine higher risk for occurrence of HPA-1a alloimmunization and FNAIT during the pregnancy.
Demographics	Year of birth, self-characterized race, ethnicity, and obstetric history. Gestational age in weeks as estimated by the principal investigator or qualified staff.
Blood samples for FNAIT laboratory testing	Pregnant participant presenting at GW 6 or after the pre-natal visit will be determined to be eligible if they are: HPA-1b/b genotype (HPA-1a negative), HLA-DRB3*01:01 positive genotype, anti-HPA-1a alloantibody negative, and fetal HPA-1a/b genotype (ie, HPA-1a positive)[a].
Screening Part 2 (after Screening Part 1 and by ~GW 16)	Consent for further assessments to confirm participants meet all inclusion/exclusion criteria and are eligible for treatment with RLYB212 and for their pregnancy to be followed according to the requirements of the protocol. Note: This is the same as "Visit 2" in Table 1-3.

FNAIT = fetal and neonatal alloimmune thrombocytopenia; GW = gestational week; SCR = screening.
[a] Two distinct samples and analyses are performed, per industry practice, to confirm absence of the HPA-1b allele in the fetus, with timing between sampling ~2 weeks.

Table 1-3 Schedule of Activities: Subjects Eligible for RLYB212 Interventional Treatment and Postnatal Follow-up

Study Period	Eligibility, Treatment, and Follow-up Period[a]																	Notes
	SV Pt 1	SV Pt 2 (B)	Initial Dose		Dose 2	Dose 3		Dose 4		Dose 5		Dose 6		Dose 7		EOS	Infant EOS	Gestational Week presented for Screening Visits, Parturition and Postpartum. Weeks since Initial Dose presented for subsequent dosing and PK sampling; visit window for Visits 4-13 are ±3 days; and ±7 for Visits 15-16
Gestational Week	**<14**	**<16**	**by 16**	**~18**	**~20**	**~24**	**~26**	**~28**	**~30**	**~32**	**~34**	**~36**	**~38**	**P[b]**	**PP4**	**PP10**		
Week Since Initial Dose			**0**	**2**	**4**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**					
Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**		
General and Safety Assessments																		
Informed consent, eligibility confirmation, demographics, and medical history	X	X																Including obstetric history. Gestational age in weeks is confirmed by the principal investigator or qualified staff
Brief physical exam (height, weight, BMI)	X	X*			X	X			X				X	X*	X	X*		X* = full physical exam Should be symptom directed
Vital signs		X	X			X				X				X		X		
12-lead ECG		X												X		X		ECG to be performed pre-dose, where applicable
AE/SAE review		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Concomitant medication review		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Obstetric and fetal doppler ultrasound		X			X	X		X		X		X	Y	X				Y = Week 38 is for high-risk pregnancies as assessed by the PI
Laboratory Assessments																		
Viral serology		X																HIV, hepatitis B and C
Hematology, chemistry, coagulation, CRP		X*			X*			X*		X*				X*	X	X		X*: Collection of sample up to 1 hour prior to study drug administration
Urinalysis		X*			X*			X*		X*				X*	X	X		X*: Collection of sample up to 1 hour prior to study drug administration

Study Period	Eligibility, Treatment, and Follow-up Period[a]																	Notes
	SV Pt 1	**SV Pt 2 (B)**	**Initial Dose**		**Dose 2**	**Dose 3**		**Dose 4**		**Dose 5**		**Dose 6**		**Dose 7**		**EOS**	**Infant EOS**	Gestational Week presented for Screening Visits, Parturition and Postpartum. Weeks since Initial Dose presented for subsequent dosing and PK sampling; visit window for Visits 4-13 are ±3 days; and ±7 for Visits 15-16
Gestational Week	**<14**	**<16**	**by 16**	**~18**	**~20**	**~24**	**~26**	**~28**	**~30**	**~32**	**~34**	**~36**	**~38**	**P[b]**	**PP4**	**PP10**		
Week Since Initial Dose			**0**	**2**	**4**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**					
Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**		
RLYB212 PK			X*	Y	X*	X*	Y	X*	Y	X*	Y	X*	Y	X*	X			X*: Collection of sample up to 1 hour prior to study drug administration. Y: Sample window of ±7 days
ADA			X*		X*	X*		X*		X*		X*		X*	X			X*: Collection of sample up to 1 hour prior to study drug administration
Study Intervention																		
Study drug administration			X		X	X		X		X		X		X*				Proposed dosing window of ±3 days. X*: Dosing within 24 hours (±24 hours) of parturition
Other Assessments																		
HPA-1a alloantibodies	X															X		
Pregnancy outcome														X				
Cord blood sample[c]														X				Platelet and hematocrit, hemoglobin, reticulocytes, total bilirubin, RLYB212 concentration. If neonatal thrombocytopenia is present, follow-up to resolution is required through normalization of platelet count as per standard of care
Newborn general status[d]														X				

Study Period	Eligibility, Treatment, and Follow-up Period[a]																	Notes
	SV Pt 1	SV Pt 2 (B)	Initial Dose		Dose 2	Dose 3		Dose 4		Dose 5		Dose 6		Dose 7		EOS	Infant EOS	Gestational Week presented for Screening Visits, Parturition and Postpartum.
Gestational Week	**<14**	**<16**	**by 16**	**~18**	**~20**	**~24**	**~26**	**~28**	**~30**	**~32**	**~34**	**~36**	**~38**	**P[b]**	**PP4**	**PP10**		Weeks since Initial Dose presented for subsequent dosing and PK sampling; visit window for Visits 4-13 are ±3 days; and ±7 for Visits 15-16
Week Since Initial Dose			**0**	**2**	**4**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**					
Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**		
Infant general status															X*		Y	X*: if infant present at 4-6 weeks. Y: 12 months (±2 weeks)

After Visit 3, windows for administering RLYB212 and other maternal assessments are ±3 days, with the exception of PK assessments at Weeks 30 and 38 which are ±7 days. The last dose of RLYB212 is to be administered within 24 hours (±24 hours) of parturition.

ADA = anti-drug antibody; AE = adverse event; BMI = body mass index; CRP = C-reactive protein; D = day; ECG = electrocardiogram; EOS = end of study; GW = gestational week; h = hour; HPA = human platelet antigen; m = minute; P = Parturition; PI = principal investigator; PK = pharmacokinetic(s); PP = postpartum; SAE = serious adverse event; SV = study visit; Tx = treatment.

[a] Screening Part 1 (presenting at GW 6 or after) women will consent to screening blood tests for HPA-1a genotyping and, if have an HPA-1b/b genotype, testing for HLA-DRB3*01:01, anti-HPA-1a alloantibody negative, and confirmation of fetal HPA-1a/b genotype via two distinct samples/analyses (as outlined in Table 1-2). At Screening Part 2, targeted after the Screening Part 1 visit, and by ~GW 16, those participants who are HPA-1b/b and HLA-DRB3*01:01 positive genotype, anti-HPA-1a alloantibody negative, and fetal HPA-1a/b genotype will be consented to further assessments to confirm they meet all inclusion/exclusion criteria and are eligible for treatment with RLYB212 and for their pregnancy to be followed according to the requirements of the protocol.

[b] Assumption is that the full term birth is at 40 weeks. If this occurs earlier than 40 weeks, the assessments planned for Week 40 will be conducted at parturition.

[c] Evaluation of neonatal thrombocytopenia will be based on the umbilical cord sample taken at parturition.

[d] Newborn general status to include Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) score. The APGAR score is to be captured twice: once at 1 minute after birth and again at 5 minutes after birth. The physical examination of the newborn is the routine clinical examination performed at birth.

Table 1-4 Schedule of Activities: Subjects Eligible for Non-interventional (Observational) Sub-study and Follow-up

Study Period	Eligibility and Follow-up Period[a]			Notes
	SV Pt 1	**SV Pt 2, *if applicable*[b]**	**EOS**	
Gestational Week	**<14**	**<16**	**PP10[c]**	
General Assessments				
Informed consent, eligibility confirmation, demographics, and obstetric history	X[δ]	X		Gestational age in weeks is confirmed by the principal investigator or qualified staff
Other Assessments				
HPA-1a alloantibodies	X		X	
Pregnancy outcome[d]			X	Based on medical records, ie, neonatal thrombocytopenia to be sourced from the neonate's medical records

BMI = body mass index; D = day; EOS = end of study; GW = gestational week; HPA = human platelet antigen; m = minute; PI = principal investigator; PP = postpartum; SV = study visit.

[δ] As defined in Table 1-2.

[a] Screening Part 1 (presenting at GW 6 or after) women will consent to screening blood tests for HPA-1a genotyping and, if have an HPA-1b/b genotype, testing for HLA-DRB3*01:01, anti-HPA-1a alloantibody negative, and confirmation of fetal HPA-1a/b genotype via two distinct samples/analyses (as outlined in Table 1-2). At Screening Part 2 targeted after the Screening Part 1 visit, and by ~GW 16, those participants who are HPA-1b/b and HLA-DRB3*01:01 positive genotype, anti-HPA-1a alloantibody negative, and fetal HPA-1a/b genotype, will be consented to further assessments to confirm they meet all inclusion/exclusion criteria and are eligible for treatment with RLYB212 and for their pregnancy to be followed according to the requirements of the protocol.

[b] In some cases, enrollment into the non-interventional (observational) sub-study will occur following SV1; therefore SV2 would not be applicable. SV2 presented for completeness.

[c] Assumption is that the live birth is at 40 weeks. If this occurs earlier than 40 weeks, the assessments planned for Week 40 will be conducted at parturition.

[d] For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

2 INTRODUCTION

RLYB212 is a recombinant human monoclonal anti-human platelet antigen (HPA)-1a immunoglobulin G (IgG)1κ antibody designed to selectively bind to HPA-1a positive fetal-derived cells or cell fragments present in the maternal circulation. The therapeutic goal of RLYB212 is to drive rapid and complete elimination of HPA-1a positive fetal antigen from the maternal circulation in HPA-1b/b pregnant women and prevent maternal HPA-1a alloimmunization (Section 2.1).

2.1 Study Rationale

A prophylactic dose regimen consisting of a single 0.12 mg subcutaneous (SC) loading dose of RLYB212 no later than gestational week (GW) 16 followed by 0.06 mg SC every 4 weeks through parturition is proposed for HPA-1b/b pregnant women at risk for HPA-1a alloimmunization. This dose regimen is the result of exposure simulations in pregnant women using a clinical pharmacology model based on pharmacokinetic (PK) and pharmacodynamic (PD) data from two clinical studies with RLYB212 in HPA-1b/b volunteers, a meta-analysis of monoclonal antibody PK parameters, allometric scaling to account for demographic differences between study populations, and the dynamic physiologic changes that occur during gestation. The dose regimen is intended to achieve near steady state RLYB212 concentrations after the first dose and to maintain RLYB212 concentrations within a target range of ~3 ng/mL to ~10 ng/mL.

The target lower boundary is supported by clinical and preclinical data demonstrating that concentrations of RLYB212 sufficient to bind ~10% of HPA-1a positive receptors in an antigen challenge model are effective to drive rapid elimination of circulating HPA-1a positive antigen positive cells. The target upper boundary of ~10 ng/mL is supported by a comprehensive toxicology program that demonstrates RLYB212 to have no observed adverse effect level (NOAEL) margins of up to 242-fold greater than 10 ng/mL in antigen negative dams bearing heterozygous antigen positive offspring (representative of HPA-1b/b women at risk of alloimmunization) and projected exposures well over 10-fold greater than 10 ng/mL in antigen positive fetuses and neonates (representative of HPA-1a/b fetuses and neonates). The target upper boundary is further informed by an extensive review of available literature on clinical assessments of alloimmunization rates and FNAIT, where maternal HPA-1a alloantibody levels of 3 IU/mL, measured at GW 28 or 34, has been reported as the threshold for the occurrence of anti-HPA-1a antibody-mediated fetal/neonatal thrombocytopenia [1, 2]. Using the most conservative estimates for binding equivalence in IU for RLYB212, the steady state exposure target accounts for an additional margin of safety by maintaining RLYB212 concentrations at or below ~0.5 IU/mL.

The purpose of this Phase 2 study is to assess the PK and safety of RLYB212 at the proposed therapeutic dose regimen in HPA-1b/b pregnant women at higher risk for HPA-1a alloimmunization and FNAIT.

Following confirmation of the prophylactic RLYB212 dose regimen, it is planned to evaluate the safety and efficacy of RLYB212 in a Phase 3 registration trial in pregnant women at higher risk for the occurrence of HPA-1a alloimmunization and FNAIT.

2.2 Background

2.2.1 Disease Background

FNAIT is a rare and potentially life-threatening disorder that can cause uncontrolled bleeding in the fetus and neonate due to maternal alloantibody-mediated destruction of fetal/neonatal platelets. It is estimated to occur in approximately 1 in 1,000 pregnancies and can result in potentially severe consequences, including fetal and neonatal intracranial hemorrhage (ICH) that can cause death of the fetus or newborn or irreversible brain damage that results in lifelong neurologic disability. ICH due to HPA-1a alloimmunization is estimated to occur in approximately 1 in 10,000 pregnancies, which translates to approximately 1,000 annual cases of ICH in Europe and North America [3].

Maternal alloimmunization to fetal platelet antigen is the prerequisite event leading to FNAIT. A fetal-maternal mismatch in HPA-1 is the most common cause of maternal alloimmunization leading to FNAIT, accounting for approximately 75% to 80% of FNAIT cases [4-7]. The HPA-1 alleles arise from a single nucleotide polymorphism that results in either a leucine (HPA-1a) or a proline (HPA-1b) at residue 33 of integrin β3 [8]. If the woman is negative for the HPA-1a antigen (HPA-1b homozygous), fetal platelets or cell fragments that are positive for paternally inherited HPA-1a antigen and which enters the maternal circulation can induce production of maternal HPA-1a alloantibodies. The maternal anti-HPA-1a alloantibodies can then traverse the placenta and destroy the fetal platelets, resulting in FNAIT [9]. Furthermore, in women who are positive for the HLA-DRB3*01:01 allele, the risk to become HPA-1a alloimmunized is approximately 25 times higher than in women who do not carry this allele [10].

There are currently no available treatments for the prevention of HPA-1a alloimmunization in pregnant women at risk for the occurrence of FNAIT. At present, most treatment modalities for pregnant women at risk for FNAIT are generally administered *post factum*, after it has been determined that the woman had a prior child born with FNAIT, and maternal alloimmunization had already occurred.

Available data suggest FNAIT has a similar pathophysiology to that of hemolytic disease of the fetus and newborn (HDFN) [11]. In the case of HDFN, a Rhesus D (RhD)-negative pregnant woman may develop alloantibodies to RhD antigens that have been inherited from the father and expressed on fetal red blood cells (RBCs). The maternal antibodies can traverse the placenta and destroy the fetal RBCs, leading to anemia and potentially hydrops fetalis, and/or death of the fetus.

Following the introduction of anti-RhD over 50 years ago [12], prophylactic administration of anti-RhD antibodies to prevent maternal RhD alloimmunization has been used with great success in the prevention of HDFN, which now rarely occurs. The anti-RhD antibody preparation (eg, WinRho SDF®; RhoPhylac®) is an IgG fraction containing a standardized antibody dose to the RhD antigen, which is administered by intramuscular injection as a prophylactic treatment to an RhD-negative pregnant woman during pregnancy at GW 27 to 28 and within 72 hours of parturition.

Since hemolytic disease of the fetus and newborn (HDFN) and FNAIT share a common alloimmune basis, administration of a prophylactic treatment is seen as the optimal approach to managing the risk of FNAIT, where passive transfer of the disease-causing agent itself (ie, anti-RhD antibodies in HDFN and anti-HPA-1a antibodies in FNAIT) can safely and effectively prevent maternal alloimmunization at concentrations well below the threshold associated with adverse clinical sequelae in the fetus or neonate. It is anticipated that RLYB212 has the potential to provide comparable efficacy for preventing HPA-1a alloimmunization and the occurrence of FNAIT, as has been established for anti-RhD products in preventing RhD alloimmunization and occurrence of HDFN.

2.2.2 Investigational Product Background

RLYB212 is a recombinant human monoclonal anti-HPA-1a IgG1κ antibody designed to selectively bind to HPA-1a positive fetal-derived cells or cell fragments present in the maternal circulation.

2.2.2.1 Nonclinical Studies

In vitro binding and activity assessments were consistent with RLYB212 being highly selective for the HPA-1a isoform of integrin β3 and having full IgG1 effector function capability. It is anticipated that RLYB212 will display no pharmacologic activity in the absence of HPA-1a and is capable of selectively opsonizing fetal-derived HPA-1a positive cells and cell fragments present in maternal circulation.

The bi-allelic nature of an HPA-1a negative pregnant woman bearing a heterozygous HPA-1a positive fetus can only be replicated in a transgenic animal model. Nonclinical efficacy and safety studies were therefore performed using a bi-allelic transgenic mouse model of FNAIT, where the gene encoding murine integrin β3 was "humanized" to reconstitute the human HPA-1a epitope by replacing 5 amino acids with their human counterparts (referred to as the APLDQ model for the amino acid substitutions made) [13].

Efficacy was assessed using an HPA-1a alloimmune challenge model where female HPA-1a negative wild-type (WT) mice received a transfusion of HPA-1a positive transgenic (APLDQ) murine platelets. Prophylactic administration of RLYB212 at doses estimated to saturate ~10% of HPA-1a platelet antigen was both necessary and sufficient to drive rapid and complete elimination of HPA-1a positive platelets in a dose-dependent manner and prevent HPA-1a alloimmunization [14].

To assess safety, general toxicology studies were conducted in WT mice to mimic dosing in antigen-negative women and in heterozygous APLDQ mice to assess exposure risks in the antigen positive fetus and neonate (with an emphasis on hematology).

A 4-week, repeat-dose, Good Laboratory Practice (GLP) toxicity study was conducted to evaluate the potential toxicity, the toxicokinetic (TK) properties, and the immunogenicity of RLYB212 in antigen negative mice. Groups of male and female C57BL/6 mice received RLYB212 SC injections at doses up to 0.125 mg/kg/dose, every 3 days for 4 weeks. RLYB212 was well tolerated with no associated toxicity, with average maximum serum concentration (C_{max}) exposures up to 532-fold higher than the target upper boundary in IPA2202.

A second 4-week repeat-dose GLP toxicity study of SC administration was conducted to evaluate the potential toxicity, the TK properties, and the potential reversibility of RLYB212 in heterozygous antigen positive mice. Groups of male and female heterozygous APLDQ mice received RLYB212 SC injections at 0.025 mg/kg once weekly. It is noted that the assay used to measure RLYB212 in circulation detects only free drug, which limits the ability to accurately assess exposure levels in antigen positive animals; therefore, a parallel TK control arm in WT mice was included for estimation of RLYB212 exposures. RLYB212 was well tolerated with average C_{max} exposure margins up to 12.5-fold over the target upper boundary in IPA2202 after the first dose and 47-fold over the target upper boundary after the last dose, with no drug-related clinical signs or effects on body weights or body weight gains. Observed effects on hematology in both antigen positive and negative animals (increase in lymphocytes, neutrophils, and total white blood cells) were considered non-adverse and were reversible.

Reproductive toxicology studies were conducted in female WT mice bred to transgenic APLDQ sires, to mimic the population of HPA-1b/b women bearing an HPA-1a positive fetus. Parallel control dose groups in pregnant mice bearing antigen-negative litters were utilized to confirm the relative ratio of maternal to fetal/neonatal exposures after dosing with RLYB212 during gestation and lactation. An additional study was also conducted in pregnant mice bearing antigen-positive litters to evaluate histopathology of heterozygous HPA-1a antigen-positive fetal-derived tissues (placenta, fetus, and neonate).

Over the series of reproductive toxicology studies that were performed, RLYB212 did not cause any adverse effects on mice during reproduction or embryo-fetal developmental (EFD) studies, ie, there were no effects on gestation, parturition, lactation, or maternal behavior in the dams and no effects on mortality, growth, or sexual maturation in their offspring. Minimal findings were noted from one study only, which included slightly increased post-implantation losses and slightly decreased fetal bodyweights in the 0.125 mg/kg dose group. Post-implantation losses in this study were variable and included several individual dams across all dose groups with losses which exceeded twice the standard deviation. Individual animal data included one dam each in the control and low dose group which were excluded due to early delivery. Decrease in fetal bodyweight was mild in the high dose group. While anti-drug antibodies (ADAs) were not measured in this study, immunogenicity could be related to these findings as RLYB212 concentrations varied and were undetectable in several animals.

This evaluation, taken together with the overall developmental and reproductive toxicity (DART) package including four other studies where no increase in post-implantation losses or decrease in pup fetal bodyweight was noted, led to an overall assessment of low risk for reproductive toxicity. Exposure margins from the reproductive toxicity package of studies ranged from average C_{max} margins in pregnant dams of up to 65-fold at gestation day 18 and 242-fold at lactation day 5. While direct measurement of RLYB212 exposure in heterozygous antigen positive offspring was not possible, antigen negative control groups demonstrated RLYB212 fetal exposures in utero at gestation day 18 of ~1-3 fold greater than those measured in the dams. Extrapolation of maternal:fetal/neonatal exposure ratios in control groups in combination with direct measurement of RLYB212 in antigen negative dams bearing antigen positive offspring enable projected exposure margins in antigen positive fetuses and neonates well over 10-fold greater than the target upper boundary of ~10 ng/mL in IPA2202. A tissue cross reactivity (TCR) study was conducted consisting of a panel of 37 adult human tissues; RLYB212 staining to the

plasma membrane of cells was generally consistent with reported expression of integrin β3 (platelets, bone marrow megakaryocytes, placental trophoblasts, and epithelial, mesothelial, and mononuclear cells). In a second TCR study evaluating fetal human tissue, RLYB212 staining was consistent with that expected, ie, endothelial cells, various epithelial cells, nerves, and mononuclear leukocytes. The remainder of binding in the fetal human tissues was cytoplasmic in nature and judged of little to no toxicologic concern due to the limited accessibility of the cytoplasmic compartment to monoclonal antibodies in vivo. Overall, the TCR studies show no significant toxicological concerns.

The nonclinical pharmacology and toxicology data support clinical investigation of RLYB212 to HPA-1b/b women bearing an HPA-1a positive fetus who are at risk for the occurrence of HPA-1a alloimmunization and FNAIT, with broad safety margins and no significant drug related toxicologic effects. Refer to the Investigational Brochure for additional nonclinical background information.

2.2.2.2 Early Clinical Development Studies

Clinical experience with RLYB212 is based on data from a Phase 1 single- and multiple-dose first-in-human study in HPA-1b/b male and female participants (IPA2001) and data from a Phase 1b single dose, prospective proof-of-mechanism (PoM)/proof-of-concept (PoC) study in HPA-1b/b males (IPA2003).

Study IPA2001 assessed the safety and PK of SC administered RLYB212 following single dose administration (0.21 mg) and multiple dose administration (0.29 mg on Day 1 followed by 0.1 mg every 2 weeks for an additional 10 weeks). RLYB212 was well tolerated in both cohorts of the study. The majority of treatment emergent adverse events (TEAEs) were reported as mild and no severe TEAEs, serious adverse events (SAEs) or TEAEs leading to withdrawal from study treatment were reported. Six possibly related TEAEs were reported in 3 participants (18.8%), all of whom received RLYB212 in the multiple dose cohort. The most common of the possibly related TEAEs was headache (2 events in 2 participants) and two of the possibly related TEAEs were of moderate severity (headache and muscle spasms). There were no clinically meaningful findings related to clinical laboratory evaluations. There were no clinically meaningful findings related to vital signs, electrocardiograms (ECGs), or physical examinations. RLYB212 displayed a PK profile consistent with an SC-administered monoclonal antibody, with a median t_{max} that ranged from 7 to 14 days, followed by a mono-exponential decline with a mean serum half-life of ~25 days. Two RLYB212 participants showed positive results for ADAs against RLYB212 (1 transient in Cohort 1 and 1 persistent with low titer in Cohort 2). No clinical findings attributable to ADAs were observed in these 2 participants and based on the similarity in PK profiles between participants with ADA positive and ADA negative results, the development of anti-RLYB212 antibodies had no clinically significant effect on the PK of RLYB212.

In Study IPA2003, a Phase 1b PoM/PoC study, the ability of RLYB212 to rapidly eliminate HPA-1a positive platelets in HPA-1b/b participants was confirmed. In this study, participants received a single SC dose of RLYB212 (0.09 or 0.29 mg) or placebo on Day 1, followed by a transfusion of HPA-1a positive platelets on Day 8. Assessments of HPA-1a positive platelet elimination were performed through Day 15 and the post treatment safety follow-up period

continued through Day 85. RLYB212 was shown to produce rapid and complete elimination of transfused HPA-1a positive platelets in HPA-1b/b participants that was dose dependent, with a reduction in mean platelet elimination half-life of ≥ 90% in both dose groups that met the prospective PoC criteria. In both dose groups, RLYB212 was well tolerated and no SAEs, severe TEAEs, or TEAEs considered related or possibly related were reported. There were no clinically meaningful findings related to clinical laboratory evaluations (including alloimmune response to HPA-1a positive platelets), vital signs, ECGs, or physical examinations. A single participant in the 0.29 mg RLYB212 group was reported to have ADA positive results on Days 15 and 29, and at the final study visit on Day 85. The ADA titer was low at each timepoint. Based on examination of the PK profiles, and PK parameter values for this single ADA positive participant remaining within the range of parameter values in the ADA negative participant at the 0.29 mg RLYB212 dose, development of anti-RLYB212 antibodies had no significant effect on the PK of RLYB212.

2.3 Benefit/Risk Assessment

2.3.1 Risk Assessment

Refer to the Investigational Brochure for detailed information on the potential benefits, risks, and reasonably expected adverse events (AEs) of RLYB212.

Table 2-1 Risk Assessment of RLYB212

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention (RLYB212)		
Maternal Risks		
Immune reactions, including ADA formation	• Since the primary sequence of RLYB212 is a native human IgG, the likelihood of immune reaction in humans is expected to be low. • RLYB212 is not predicted to have any inherent pharmacologic activity against host tissues in HPA-1b/b pregnant women, as these individuals do not carry the HPA-1a antigen. • Off-target, nonspecific risks of any biologic or monoclonal antibody such as hypersensitivity reactions may be observed. • Clinical manifestation of hypersensitivity reactions may range from immediate onset findings such as rash, urticaria, and in more severe cases, anaphylaxis, to delayed onset finding such as influenza-like symptoms (pyrexia, sore throat).	• Measures implemented to monitor the risk of immune reactions: ○ Monitoring of participant safety (eg, occurrence of TEAEs). ○ Participants observed in clinic for 1 hour after SC administration of RLYB212. ○ ADA monitored per SoA (Section 1.3 and Section 8.7). • Impact of ADAs on the PK of RLYB212 will be evaluated. • Excipients used in the RLYB212 drug product are commonly used with mAbs; ○ The PS 80 concentration is less than chronically used drug products such as adalimumab and rituximab, ○ Additionally, precautions are made in the protocol to dose at the investigative site, with post-dose observation for 1 hour.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	• ADA may develop after administration of RLYB212. ADAs have been reported in three participants in Phase 1 studies (IPA2001 and IPA2003), two participants after single dose and one participant after multiple dose administration. The development of anti-RLYB212 antibodies had no clinically significant effect on the PK of RLYB212. • Repeat administration of polysorbate (PS) 80 containing drug may induce anaphylaxis, which causes uterine contractions or malperfusion of the uterus with fetal outcomes.	
Injection site reaction	• Smaller SC injection volume of RLYB212 may reduce likelihood of injection site reactions. • Symptoms of injection site reaction may include pain, swelling, erythema, pruritus, and rash around the site of injection.	• SC injection will be administered by the investigator or designee. • Participants will be observed in clinic for 1 hour after administration of RLYB212. • Injection site rotation may be considered to help avoid soreness at previous SC injection site(s). • Participant safety will be monitored throughout the study.
Study Participation		
Risks related to study procedures	• Study specific procedures are those used in obstetric care. Some assessments (eg, blood draws or obstetrical doppler ultrasounds) may be more frequent.	• Participants will be monitored per obstetric standard of care
Fetal and Neonatal Risks		
Fetal	• Occurrence of fetal thrombocytopenia due to presence of high circulating fetal levels of RLYB212. • Potential adverse fetal outcomes including spontaneous abortion, preterm delivery, intrauterine growth retardation, and hemorrhage. • Placental inflammation or compromise from RLYB212 binding to fetal antigen on placenta. • Exposure of an HPA-1b/b fetus to RLYB212	• The safety of the RLYB212 SC dose regimen is supported by general toxicology studies and developmental and reproductive toxicology studies (including histological examination). Across studies, projected exposure margins in antigen positive fetuses and neonates (representative of HPA-1a/b fetuses and neonates) were well over 10-fold greater than the target upper boundary of 10 ng/mL). • The target upper boundary for RLYB212 exposure (~10 ng/mL) is ~6-fold below the safety threshold for anti-HPA-1a alloantibody titers associated with clinical sequelae of FNAIT, as reported by Killie et al [1].

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	• Cell-free fetal DNA collected for fetal genotyping is at low levels before GW 10 (<3% of total maternal circulating cfDNA and gradually increases by about 1% by Week 20). [15] Due to these low levels of fetal DNA prior to GW 16, confirmation of fetal HPA-1 genotype may come after initial dose of RLYB212 at GW 16. (Section 8.1.1 and Section 8.1.2). Consequently, some participants carrying a potentially HPA-1b/b fetus will be exposed to the loading dose of RLYB212 despite not being at risk for alloimmunization.	• The Phase 2 study (IPA2202) employs sentinel subject dosing through pregnancy followed by two sequential, sequenced cohorts. Modification of the RLYB212 dose regimen may be performed during the study based on available PK data from participant(s) with a completed pregnancy, to ensure desired steady state concentrations are maintained through pregnancy and parturition between a target lower bound of ~3 ng/mL and a target upper bound of ~10 ng/mL. • For women receiving RLYB212 loading dose (0.12 mg) before the fetal genotype is definitively determined, there is a ~85% chance that the fetus will be HPA-1a/b and hence express the antigen (due to the prevalence of the HPA-1b gene and application of the Hardy-Weinberg principle in the case of unknown paternal genotype). In that case, all aspects to mitigate fetal/placental harm noted above will be in place and the loading dose will have been administered appropriately. There remains a roughly 15% chance, however, that the fetus will be determined to be HPA-1b/b, consistent with the maternal genotype. In this case, RLYB212 administration will be stopped, and the pregnancy will be followed for an additional 4 weeks for safety and PK assessment. Since RLYB212 is directed against the HPA-1a epitope, which neither mother nor fetus express, the antibody will not bind to maternal or fetal tissues. Therefore, RLYB212 is expected to pose no risk other than that of any exogenous monoclonal antibody, as outlined under “Maternal Risks” within this table. • Fetal doppler ultrasound monitoring will be performed at regular intervals during the study and consistent with obstetric standard of care, to detect any evidence of placental structural abnormality, organ pathology, hemorrhage, or delayed growth in the fetus. • A cord blood sample will be collected at delivery to evaluate fetal exposure to RLYB212.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		• The study design employs sentinel subject dosing through pregnancy followed by 2 sequential, sequenced cohorts. Maternal PK at the end of pregnancy will be evaluated before initiating dosing in the subsequent cohort (Section 4.1). • An independent DMC will monitor safety data at regular intervals throughout the duration of the study according to a study specific DMC charter (Section 2.3.4 and Section 10.1.5 (Appendix 1).
Neonate/infant	• Potential risks related to continued presence of circulating RLYB212 in neonatal/infant circulation or breast milk. • It is expected that transient levels of RLYB212 will be present during the first few weeks of breast feeding. • Based on experience in individuals with FNAIT, breast feeding has not affected the rapid recovery of platelet counts after birth (unlike the situation with ITP) [16] and hence due to its benefits, breast feeding is recommended by the ICTMG FNAIT patient information leaflet. • Levels of anti-HPA-1 activity transmitted by breast milk during the peripartum period in the case of RLYB212 are anticipated to be considerably less than those transmitted to infants with active FNAIT.	• A cord blood sample will be collected at delivery to assess for possible occurrence of neonatal thrombocytopenia. • Safety of the neonate/infant up to 4-6 weeks postpartum will be assessed by neonatal infant standard of care practices including neonatal/infant general status examination, measurement of APGAR score (at birth). • The benefit of breast feeding outweighs the potential risk; breast feeding will be permitted, therefore no mitigation is required.

ADA = anti-drug antibody; AGPAR = Appearance, Pulse, Grimace, Activity, and Respiration C_{max} = maximum serum concentration; DMC = data monitoring committee; FNAIT = fetal and neonatal alloimmune thrombocytopenia; HPA = human platelet antigen; ICTMG = International Collaboration for Transfusion Medicine Guidelines; IgG = immunoglobulin G; ITP = immune thrombocytopenia; mAb = monoclonal antibody; PK = pharmacokinetic; SC = subcutaneous; SoA = schedule of activities; TEAE = treatment-emergent adverse events.

2.3.2 Benefit Assessment

The target population for prophylactic antenatal administration of RLYB212 is pregnant women who are at higher risk for the occurrence of HPA-1a alloimmunization and FNAIT in their pregnancy. There is currently no available prophylactic treatment to prevent HPA-1a alloimmunization. While the ability of RLYB212 to prevent HPA-1a alloimmunization will be assessed in a planned Phase 3 registration trial, available nonclinical and clinical data support potential therapeutic benefit, based on:

- A similar condition, HDFN, is effectively prevented by prophylactic treatment with small doses of antigen specific IgG (ie, anti-RhD antibody preparations).

- RLYB212 prevents HPA-1a alloimmunization at the same doses that produce accelerated elimination of HPA-1a positive platelets in the bi-allelic APLDQ murine model of FNAIT.

- It is noted that the effective concentrations shown to prevent alloimmunization are well below the NOAEL for RLYB212 in the general and reproductive toxicology studies.

- RLYB212 has demonstrated rapid and complete elimination of HPA-1a/b platelets exogenously administered to participants with an HPA-1b/b genotype.

- Clinical data support a favorable tolerability profile of RLYB212 at exposures in HPA-1b/b non-pregnant volunteers that exceed the ~10 ng/mL target upper boundary by ~2-fold, while the target upper boundary is expected to provide a 6-fold safety margin for the fetus.

2.3.3 Overall Benefit-Risk Conclusion

The benefit-risk of RLYB212 is assessed in the context of the target population of pregnant women who are at higher risk for the occurrence of HPA-1a alloimmunization in their pregnancy and for which no prophylactic therapeutic presently exists. The intended dose regimen is projected to maintain steady state concentrations of RLYB212 that are well below those evaluated in relevant nonclinical investigations including maternal-fetal toxicology data and the threshold level of native HPA-1a alloantibodies associated with risk of fetal/neonatal thrombocytopenia reported by Killie et al. [1] (Section 4.3). The RLYB212 dose regimen may also be modified during the study based on available PK data from participant(s) with a completed pregnancy, to ensure desired steady state concentrations are maintained through pregnancy and parturition between the target lower bound of ~3 ng/mL and the target upper bound of ~10 ng/mL.

In considering all measures to be implemented to closely monitor and manage potential risks identified in association with RLYB212, it is the Sponsor's assessment that the benefit/risk for administration of RLYB212 to pregnant women is favorable and supports inclusion of eligible participants in accordance with the protocol-defined eligibility requirements.

2.3.4 Other Risk Minimization Actions/Monitoring

An independent data monitoring committee (DMC) will monitor safety outcomes and study conduct and will provide recommendations for consideration by the Sponsor regarding the continuation of the study, dosing of further participants in the study, or stopping the study for all participants or subgroups of participants in the study. Safety data will include, but not be limited to, those outcomes listed as primary and secondary endpoints.

A detailed DMC charter and operating rules will be prepared and signed by DMC members before study start. Additional information on the DMC is provided in Section 10.1.5 (Appendix 1).

3 OBJECTIVES AND ENDPOINTS

3.1 Interventional

Objective	Endpoint
Primary	
• To evaluate the PK profile of RLYB212 during pregnancy following repeat SC administration	• Maternal PK parameters, eg, ○ half-life of RLYB212 ($t_{½}$) ○ maximum RLYB212 concentration (C_{max}) ○ time to maximum RLYB212 concentration (t_{max}) ○ apparent clearance (CL/F) of RLYB212 ○ apparent volume of distribution (Vd) ○ area under the RLYB212 concentration versus time curve (AUC)
• To assess maternal and fetal safety of RLYB212 during pregnancy	• Type, seriousness, and incidence of AEs • Physical examination findings • Vital signs • Maternal clinical laboratory values • ECG • Obstetric/fetal doppler ultrasound
Secondary	
• To evaluate RLYB212 exposure in the neonate at delivery	• Concentration of RLYB212 in cord blood
• To assess the safety of RLYB212 in the HPA-1a positive neonate	• Type, seriousness, and incidence of AEs including fetal/neonatal AEs • Physical examination findings, including APGAR scores • Vital signs
• To assess the immunogenicity of RLYB212	• Development of ADAs
• To assess pregnancy and neonatal outcomes following antenatal RLYB212 administration	• Number of spontaneous abortions • Number of elective abortions • Number of stillbirths • Number of premature births • Number of full term births (≥ 37 completed weeks of gestation) • Overall health and development of infants at 4-6 weeks and 12 months of age
• To assess the occurrence of neonatal thrombocytopenia following antenatal RLYB212 administration	• Neonatal thrombocytopenia[a] and severe neonatal thrombocytopenia
• To assess the occurrence of HPA-1a alloimmunization	• Presence of maternal anti-HPA-1a alloantibodies at Week 10 post pregnancy[b]

ADA = anti-drug antibody; AE = adverse event; APGAR = Appearance, Pulse, Grimace, Activity, and Respiration; ECG = electrocardiogram; HPA-1a = human platelet antigen 1a; PK = pharmacokinetic(s); SC = subcutaneous.

[a] Evaluation of neonatal thrombocytopenia will be based on the umbilical cord sample taken at parturition.

[b] For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

3.2 Non-Interventional (Observational)

Objective	Endpoint
• To inform the frequency of HPA-1a alloimmunization among pregnant women identified at higher FNAIT risk	• Occurrence of anti-HPA-1a maternal alloimmunization at Week 10 postpartum[a]
• To inform the frequency of pregnancy outcomes among pregnant women identified at higher FNAIT risk	• Rate of spontaneous abortion, defined as non-deliberate fetal death which occurs prior to 19 weeks of gestation • Rate of elective abortion, defined as deliberate termination of pregnancy at any time in gestation • Rate of stillbirth, defined as non-deliberate fetal death anytime in gestation on or after 19 weeks of gestation • Rate of premature birth, defined as live birth prior to 37 completed weeks of gestation • Rate of full term birth (≥ 37 completed weeks of gestation)
• To inform the frequency, where data are available, of neonatal thrombocytopenia in infants born to women who have alloimmunized, as determined by detectable anti-HPA-1a antibody at Week 10 postpartum[a]	• Neonatal thrombocytopenia and severe neonatal thrombocytopenia, as determined by a platelet count $< 150 \times 10^9$/L and $< 50 \times 10^9$/L, respectively, where data are available

[a] For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

4 STUDY DESIGN

4.1 Overall Design

This study is a single-arm, open-label, multicenter study of RLYB212 in HPA-1b/b pregnant women who are at higher risk for the occurrence of HPA-1a alloimmunization and FNAIT. A laboratory testing paradigm will be applied at screening to identify women at higher risk for HPA-1a alloimmunization.

The study is comprised of three phases: a two-part screening phase, an antenatal treatment phase, and a postpartum follow-up phase. An overview of the study design is provided in Figure 1-1 and Section 1.2. There will be a non-interventional (observational) sub-study for those participants who do not receive RLYB212 (Figure 1-1).

An independent DMC will monitor safety data at regular intervals throughout the duration of the study according to a study specific DMC charter. Study duration for each participant is anticipated to be ~44 weeks, inclusive of the screening visits through the Week 10 postpartum visit. For each child born during the study, the duration is ~12 months.

The study has been designed to enroll an initial sentinel participant followed by staged enrollment of participants to two sequential cohorts. The single sentinel participant will be enrolled and receive SC administered RLYB212 for the scheduled duration of their pregnancy. The DMC will review all available data through parturition for this sentinel participant, together with RLYB212 exposure data and available safety data in the neonate. This review will be performed before commencing dosing in subsequent participants. Upon completion of the DMC review for that sentinel participant, a cohort of up to 3 additional participants will be enrolled and receive SC administration of RLYB212. Following DMC review of all available data through parturition for the additional 3 participants, together with RLYB212 exposure data and safety data in the neonates, a second cohort of the final 4 participants will be enrolled.

Modification of the RLYB212 dose regimen may be necessary to ensure steady state concentrations are maintained through pregnancy and parturition between a target lower bound of ~3 ng/mL and a target upper bound of ~10 ng/mL. Adjustment of the RLYB212 dose/dose frequency may be made within a cohort or between cohorts, based on available PK data from participant(s) with a completed pregnancy. If the Sponsor determines it is necessary to adjust the RLYB212 dose regimen for future participants, the Sponsor will communicate this to the investigator and study staff. Dose modifications are not anticipated during the treatment period of an individual participant.

A non-interventional (observational) sub-study (IPA2202A) is planned based on the phased approach to participant enrollment that results from the sequential cohort design. Because of the rarity of the potential occurrence of HPA-1 alloimmunization, the non-interventional (observational) sub-study will serve to augment an ongoing FNAIT Natural History Study (NCT05345561), ie, generating natural history data on the occurrence of HPA-1a alloimmunization and pregnancy/neonatal outcomes in women at higher FNAIT risk.

No formal interim analyses will be conducted for this study. Preliminary data may be used for summary purposes and for communication with health authorities.

4.2 Scientific Rationale for Study Design

The dose regimen to achieve therapeutic exposure levels of RLYB212 is informed by a clinical pharmacology model that incorporates PK data from a first-in-human safety and PK study (IPA2001) in HPA-1b/b participants, and PK and PD data from a Phase 1b platelet elimination PoM/PoC study (IPA2003) in HPA-1b/b participants. The clinical pharmacology model also incorporates projected PK parameters from a meta-analysis of monoclonal antibodies, allometric scaling to account for demographic differences between study populations, and the dynamic physiologic changes that occur during gestation. The purpose of this Phase 2 study is to assess the PK and safety of RLYB212 at the proposed therapeutic dose regimen in HPA-1b/b pregnant women at higher risk for HPA-1a alloimmunization and FNAIT and to guide appropriate dosing for a planned Phase 3 safety and efficacy registration trial.

To achieve the study's objective, a single arm, open-label design is utilized with all participants receiving RLYB212. The enrolled study participants represent the target population for RLYB212 ie, HPA-1b/b pregnant women bearing an HPA-1a positive fetus, who are at higher risk for the occurrence of HPA-1a alloimmunization and FNAIT. It is planned to follow 8 participants for whom the pregnancy results in a live birth to enable the PK and preliminary safety profile of RLYB212 to be evaluated during pregnancy and to guide appropriate dosing for the planned Phase 3 registration trial.

4.3 Justification for Dose

Prophylactic treatment with SC dosing of RLYB212 is designed to drive rapid and complete elimination of HPA-1a positive fetal-derived cells and cell fragments from the maternal circulation, thereby preventing HPA-1a maternal alloimmunization, by maintaining the highest safe exposure of RLYB212 throughout the entire second and third trimesters and immediately following parturition. Additionally, by administering RLYB212 via SC doses, peak to trough fluctuations are minimized and incorporating an initial 2 × loading dose is projected to achieve near steady state concentrations after the first dose, thereby enabling a more consistent PK profile over the entire treatment period.

A prophylactic dose regimen consisting of a single 0.12 mg SC loading dose of RLYB212 followed by 0.06 mg SC Q4W is proposed as the initial dose for HPA-1b/b pregnant women at risk for HPA-1a alloimmunization. The dose regimen is intended to achieve near steady state RLYB212 concentrations after the first dose and maintain them within a target range of ~3 ng/mL to ~10 ng/mL The dose regimen will be adjusted empirically as additional subjects are enrolled to achieve the target range. It is possible that additional RLYB212 will be necessary due to target mediated drug disposition (TMDD), ie, RLYB212 binding to the HPA-1a epitope expressed on placental proteins.

The target lower boundary is supported by clinical and preclinical data demonstrating that concentrations of RLYB212 sufficient to bind ~10% of HPA-1a positive receptors in an antigen

challenge model are effective to drive rapid elimination of circulating HPA-1a positive antigen positive cells.

The target upper boundary of ~10 ng/mL is supported by a comprehensive toxicology program that demonstrates RLYB212 to have NOAEL margins of up to 242-fold greater than 10 ng/mL in antigen negative dams bearing heterozygous antigen positive offspring (representative of HPA-1b/b women at risk of alloimmunization) and projected exposures well over 10-fold greater than ~10 ng/mL in antigen positive fetuses and neonates (representative of HPA-1a/b fetuses and neonates). The target upper boundary is further informed by an extensive review of available literature on clinical assessments of alloimmunization rates and FNAIT, where maternal HPA-1a alloantibody levels of 3 IU/mL, measured at GW 28 or 34, has been reported as the threshold for the occurrence of anti-HPA-1a antibody-mediated fetal/neonatal thrombocytopenia [1, 2]. These data have been used as a basis for our selection of an anti-HPA-1a value of 3 IU/mL as an empirically supported maximum threshold for calculating a margin of safe exposure during pregnancy, with a negative predictive value of 95% (95% CI: 86-98%) in the prevention of anti-HPA-1a antibody mediated fetal/neonatal thrombocytopenia, as reported in Killie et al. [1]. Using the most conservative estimates for binding equivalence in IU for RLYB212, the steady state exposure target accounts for an additional margin of safety by maintaining RLYB212 concentrations at or below ~0.5 IU/mL.

Based on the NOAEL exposure margins in the RLYB212 non-clinical safety toxicology program and by adopting the most conservative estimate of RLYB212 binding equivalence in IU as it compares to the World Health Organization standard for anti-HPA-1a antibodies, it is estimated that the proposed dosing regimen would be well within safe limits for the developing fetus while maintaining the capacity to drive rapid and complete elimination of fetal antigen positive cells or cell fragments entering into the maternal vascular compartment.

Refer to the Investigational Brochure for additional background information.

4.4 End of Study Definition

An interventional participant is considered to have completed the study if they have completed all phases of the study, including the visit at 10 weeks postpartum or at 10 weeks from the date of the pregnancy terminating event (ie, abortion [spontaneous/elective] or stillbirth). A child born during the study will have completed after the 12-month visit has been completed.

A non-interventional (observational) sub-study participant is considered to have completed the study if they have completed all phases of the study, including the visit at 10 weeks postpartum or at 10 weeks from the date of the pregnancy terminating event (ie, abortion [spontaneous/elective] or stillbirth).

The end of the study is defined as the date of the last visit of the last participant in the study or last scheduled procedure shown in the schedule of activities (SoA [Section 1.3]) for the last participant in the trial (globally).

5 STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

Eligibility for this study will be established using a two-part screening procedure. Screening Part 1 will identify those participants who are at higher risk for the occurrence of maternal HPA-1a alloimmunization and FNAIT in their pregnancy. If a participant meets eligibility criteria in Screening Part 1, they may proceed to Screening Part 2 to determine their eligibility for enrollment and subsequent treatment with RLYB212.

Eligibility criteria are provided in Section 5.1 and Section 5.2.

Approximately 5,000 participants will be screened to identify 8 pregnant individuals who meet the study-specific blood test results required for study inclusion and for whom the pregnancy results in a live birth and who are evaluable for PK and safety assessments. Women in their first or subsequent pregnancy may be included in the study. These participants will be HPA-1b/b and HLA-DRB3*01:01 positive genotype, anti-HPA-1a alloantibody negative, and fetal HPA-1a/b positive.

5.1 Eligibility Criteria for Screening (Part 1)

The following criteria will be assessed.

5.1.1 Inclusion Criteria to be Confirmed at Screening Part 1

1. Written/signed informed consent (for Screening Part 1)
2. 18-45 years of age
3. Pregnant women who present at GW 6 or after and confirmed to be:
    a. HPA-1b/b (HPA-1a negative)
    b. HLA-DRB3*01:01 positive
    c. Anti-HPA-1a alloantibody negative
    d. Carrying an HPA-1a/b (HPA-1a positive) fetus

5.1.2 Exclusion Criteria to be Confirmed for Screening Part 1

1. Prior history of HPA-1a-related FNAIT
2. Prior history of HPA-1a alloimmunization
3. Prior history of platelet transfusion or other blood transfusions
4. BMI ≥ 35 kg/m^2

5.2 Eligibility Criteria for Study Intervention

Participants who meet all eligibility criteria in Screening Part 1 (ie, HPA-1b/b, HLA-DRB3*01:01 positive genotype; anti-HPA-1a alloantibody negative; fetal HPA-1a/b genotype) and who provide informed consent for Screening Part 2, will be assessed for eligibility to enter the study's antenatal treatment phase and to receive treatment with RLYB212 provided all inclusion and no exclusion criteria are met. If a participant is eligible for the intervention (Screening Part 2), but the current cohort has been fully enrolled, the participant will be requested to consent for enrollment in the non-interventional (observational) sub-study.

5.2.1 Inclusion Criteria for Study Drug Administration to be Confirmed at Screening Part 2

1. Written/signed informed consent (for Screening Part 2)
2. 18-45 years of age
3. Pregnancy no later than GW 16 at the time of first study drug administration as determined by the principal investigator or qualified staff

5.2.2 Exclusion Criteria for Study Drug Administration to be Confirmed at Screening Part 2

Participants are excluded from the study if any of the following criteria apply:

1. Multiple pregnancy (>1 fetus)
2. Known sensitivity and/or immediate hypersensitivity to any components of RLYB212 or its formulation
3. History of hypersensitivity to protein therapeutics
4. Any participant with a history of past or current intravenous immunoglobulin (IVIG) use for an underlying disease (eg, autoimmune disease)
5. Requires ongoing systemic corticosteroid or systemic immunosuppressive treatment for any reason
6. History of pre-eclampsia in a previous pregnancy
7. Participants with an in vitro fertilized pregnancy or serving as pregnancy surrogates
8. Current (or within the last 90 days) participation in another clinical study and have received an investigational drug and device within the last 90 days
9. Participants known or suspected of not being able to comply with this study protocol
10. History of any connective tissue or autoimmune or autoinflammatory disease, especially autoimmune thrombocytopenia

11. Any co-morbid medical or obstetric condition(s), laboratory abnormality, concomitant treatment, or other reason that, in the investigator's opinion, could adversely affect the safety of the participant and/or fetus, impair the assessment of study results, or preclude compliance with the study

5.3 Lifestyle Considerations

No dietary or lifestyle restrictions are required.

5.4 Screen Failures (Screening Part 2)

Screen failures are defined as participants who consent to participate in the clinical study but do not subsequently receive the study intervention. A minimal set of screen failure information for those failing to meet criteria at Screening Part 1 and Screening Part 2 is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, assessment of eligibility prior to enrollment, and any SAEs.

Pregnant individuals who do not meet the eligibility criteria for RLYB212 administration may not be rescreened during their current pregnancy. However, these individuals will be requested to consent for the non-interventional (observational) sub-study (Figure 1-1 and Table 1-1).

6 STUDY INTERVENTION

The study intervention, RLYB212, will be administered to participants in accordance with the study protocol.

Refer to the Pharmacy Manual for detailed information regarding the storage, preparation, administration, and destruction of RLYB212.

6.1 Study Intervention(s) Administered

Dose, formulation, and administration instructions for RLYB212 are provided in Table 6-1. Reference is made to Section 6.5 regarding the approach to dose modification within or between cohorts.

Table 6-1 Study Intervention(s) Administered

	RLYB212
Dosage formulation	Vials, each containing 1 mL of RLYB212 (0.2 mg/mL) solution for SC injection
Unit dose strength(s)/dosage level(s) and dosage frequency[a]	RLYB212 is to be administered at an initial (loading) dose of 0.12 mg no later than GW 16 followed by maintenance (repeat) doses of 0.06 mg Q4W throughout the pregnancy and with a final 0.06 mg dose within 24 hours (±24 hours) of parturition.
Route of administration	SC injection using vial and syringe
Dosing instructions	Refer to Pharmacy Manual for details. The participant will be carefully observed during the injection and observed for 1 hour after the administration.
Storage	2 °C to 8 °C (do not freeze)

GW = gestational week; Q4W = every 4 weeks; SC = subcutaneous.
[a] Refer to Section 4.3 for justification of dose/dosing frequency.

6.2 Preparation/Handling/Storage/Accountability

The investigator or designee must confirm appropriate temperature and other storage conditions have been maintained during transit for all study materials received and any discrepancies are reported and resolved before use of the study intervention.

Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records). Further guidance and information for the final disposition of unused study interventions are provided in the Pharmacy Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study and there is no randomization or blinding.

6.4 Study Intervention Compliance

Participants will be dosed at the site and receive RLYB212 directly from the investigator or designee and will be observed for 1 hour after dosing by the investigator/site staff. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the case report form (CRF). The dose of RLYB212 and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

6.5 Dose Modification

Modification of the RLYB212 dose regimen may be necessary to ensure steady state concentrations are maintained through pregnancy and parturition between a target lower bound of ~3 ng/mL and a target upper bound of ~10 ng/mL.

Modification of RLYB212 dose/dose frequency may be made within a cohort or between cohorts, based on available PK data from participant(s) with a completed pregnancy. If the Sponsor determines it is necessary to adjust the RLYB212 dose regimen for future participants, the Sponsor will communicate this to the Investigator and study staff.

Dose modifications are not anticipated during the treatment period of an individual participant.

6.6 Concomitant Therapy

Standard treatments given during obstetric care are allowed. Routine vaccines, given at recommended times during or immediately after pregnancy, will be allowed in the study.

Any medication (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) or other specific categories of interest that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Concomitant medications may be considered on a case-by-case basis by the investigator in consultation with the medical monitor.

6.6.1 Rescue Medicine

There are no rescue medications defined in the protocol.

6.6.2 Excluded Treatments, Medical Devices, and/or Procedures During Study Period

Broad spectrum immunosuppressant agents and IVIG are not allowed as concomitant medications with RLYB212 administration. If such treatments are required, RLYB212 will be discontinued but the participant will continue to be followed as described in Section 7.1.

6.7 Intervention of Overdose or Dosing Errors

In the event of an error in dosing, including overdose, the investigator should contact the medical monitor/Sponsor immediately and closely monitor the participant for any AE/SAE and laboratory abnormalities. Where available, the quantity of the excess dose as well as the duration of the overdose should be documented. The Sponsor will determine when and if further dosing will be given, and what dose.

7 DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

In rare instances, it may be necessary for a participant to permanently discontinue (definitive discontinuation) study intervention. If study intervention is definitively discontinued, the participant will remain in the study to be evaluated for pregnancy outcomes and postnatal follow-up in the event of live birth, and for anti-HPA-1a antibody determination. If treatment is discontinued because a delayed cell-free fetal DNA determination reveals the fetus is HPA-1b/b, the subject will also return 4 weeks after RLYB212 dosing for a safety visit, the context of which will the same as Visit 5 in the SoA (Section 1.3). Participants withdrawing consent to be in the study or administrative reasons for discontinuation should proceed directly to the end of study (EOS) visit (Week 10 postpartum), if possible.

Refer to the SoA (Section 1.3) for details of the data to be collected at the time of discontinuation of RLYB212, follow-up, and for any further evaluations that need to be completed. Permanent discontinuation of RLYB212 for safety-related reasons will not be followed by a rechallenge.

Information on the study assessments and procedures is provided in Section 8.

7.1.1 Temporary Discontinuation

In the event of temporary discontinuation of study intervention, the investigator should contact the medical monitor/Sponsor immediately. Further course of action will be decided upon by discussion between investigator, Sponsor, and with the DMC, as needed.

7.1.2 Study Stopping Rules

In the event of maternal death[1], treatment will be withheld for all study participants, pending Sponsor evaluation and DMC recommendation.

Following occurrence of a maternal/fetal/neonatal common terminology criteria for adverse events (CTCAE) Grade 4[1] or Grade 5 event as specifically described by Spencer et al [17] or any of the fetal/neonatal AEs specified in Section 10.3.3 (Appendix 1), treatment with RLYB212 will not be initiated in any new study participants pending Sponsor evaluation and DMC recommendation. Reference is made to Section 10.3.3 (Appendix 1) for guidance on using the maternal and fetal AE severity grading criteria.

If one or more fetus or neonate in the study has evidence of severe thrombocytopenia or hemorrhage, RLYB212 administration will be withheld for all participants pending Sponsor evaluation and DMC recommendations.

[1] Events that are definitely not related to study treatment (eg, accidents or other external causes) will not trigger study-stopping rules.

If 2 study participants have stopped RLYB212 administration for any safety-related reason, RLYB212 administration will be withheld for all participants pending Sponsor evaluation and DMC recommendation.

In all cases in which dosing of RLYB212 is suspended (for an individual participant or for all participants), the Sponsor and DMC will complete an evaluation and decide on further dosing promptly. This is so that dosing can be resumed, if appropriate, without serious disruption of RLYB212 coverage.

7.2 Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at their own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons.

Participants for whom treatment is withdrawn but who retain consent to continue in the study should be followed through all activities in the study SoA (Section 1.3). Those who withdraw consent should have EOS (Week 10 postpartum) procedures performed as their end of study visit.

If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

Any participants discontinuing the study early for reasons unrelated to pregnancy outcomes or safety will be replaced.

Up to 4 participants may be replaced if they were assigned to study drug but did not receive the study drug. Participants may also be replaced for reasons other than clinical safety, including but not limited to insufficient PK sample collection to evaluate the PK profile of RLYB212 during pregnancy.

In addition, where the outcome of the pregnancy is not a live birth, these participants may be replaced. Any decision regarding the replacement of participants will be discussed with the DMC prior to additional enrollment of participants into the study.

7.3 Lost to Follow-up

A participant will be considered lost to follow-up if the participant repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.

- Should the participant continue to be unreachable, she will be considered to have withdrawn from the study.

Discontinuation of specific sites or of the study as a whole are addressed in Section 10.1 (Appendix 1).

7.4 Non-interventional (Observational) Sub-study – Subject Decision to Withdraw

Participants have the right to withdraw from the study at any time and for any reason without prejudice to their future medical care by the physician or at the institution. Withdrawal of consent for a study means that the participant does not wish to or is unable to continue further study participation. Participant data up to withdrawal of consent will be included in the analysis of the study and, where permitted, publicly available data can be included after withdrawal of consent. The investigator is to discuss with the subject appropriate steps for withdrawal of their consent from the study.

8 STUDY ASSESSMENTS AND PROCEDURES

Interventional study procedures and their timing are summarized in the SoA (Section 1.3, Table 1-3). Non-interventional (observational) sub-study procedures and their timing are summarized in the SoA (Section 1.3, Table 1-4). Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA (Section 1.3), is essential and required for integrity of study conduct.

Information on how samples are to be stored, processed, and shipped is provided in the Laboratory Manual.

8.1 FNAIT Laboratory Screening Assessments

In participants presenting at GW 6 or after who provide informed consent, blood samples will be collected at the Screening Part 1 visit to assess for higher risk for the occurrence of HPA-1a alloimmunization and FNAIT during their pregnancy.

The first test will be a test for maternal HPA-1 genotype. In participants identified as HPA-1b/b (ie, HPA-1a negative), subsequent tests will be performed to confirm the participant has an HLA-DRB3*01:01 positive genotype, is anti-HPA-1a alloantibody negative, and is carrying an HPA-1a/b (ie, HPA-1a positive) fetus.

8.1.1 Screening Part 1

The screening test paradigm is provided in Table 8-1.

Table 8-1 Screening Test Paradigm

Test No.	Test	Description		Result	Screening Specific Action
1	Maternal HPA-1 genotype	Test will identify women who are HPA-1b/b, indicating risk for HPA-1a sensitization and occurrence of FNAIT	**1a**	HPA-1b/b (HPA-1a negative)	Perform Test **2** and **3**
			1b	HPA-1a/b; HPA-1a/a	None – no further testing (ineligible)
2	Maternal HLA-DRB3*01:01 genotype [δ]	Test will identify women who are HLA-DRB3*01:01 positive, indicating a higher alloimmunization risk; performed concurrently with Test 3	**2/3a**	HLA-DRB3*01:01 positive, Anti-HPA-1a antibody negative	Perform Test **4**
3	Maternal anti-HPA-1a antibody [δ]	Test will establish women who have no detectable anti-HPA-1a antibody; performed concurrently with Test 2	**2/3b**	HLA-DRB3*01:01 positive, Anti-HPA-1a antibody positive	None – no further testing (ineligible)
			2/3c	HLA-DRB3*01:01 negative, Anti-HPA-1a antibody negative	None – no further testing (ineligible)
4	Fetal HPA-1 genotype	A cffDNA test to inform on the presence of the antigenic stimulus for maternal alloimmunization	**4a**	HPA-1a/b	Treat with SC RLYB212 and follow up for final assessments
			4b	HPA-1b/b	None – no further testing (ineligible)

cffDNA = cell-free fetal DNA; FNAIT = fetal and neonatal alloimmune thrombocytopenia; HPA = human platelet antigen
[δ] Note: for operational efficiency, Test 2 and Test 3 are performed simultaneously

Screening Part 1 is an initial visit that includes the informed consent, demographic questioning (including obstetric history), and the collection of a blood sample for FNAIT testing (Table 1-2). This visit is to take place no earlier than GW 6. Individuals will consent to HPA-1 genotyping (Test 1) and, if HPA-1b/b, subsequent screening for HLA-DRB3*01:01 (Test 2), anti-HPA-1a antibodies (Test 3), and confirmation of fetal HPA-1a genotype (Test 4), as required.

Two distinct samples and analyses are performed, per industry practice, to confirm absence of the HPA-1b allele in the fetus, with timing between sampling ~2 weeks.

8.1.2 Screening Part 2

Participants who meet all eligibility criteria in Screening Part 1 (SoA, Table 1-3, ie, HPA-1b/b and HLA-DRB3*01:01 positive genotype; anti-HPA-1a alloantibody negative; fetal HPA-1a/b genotype) and who provide informed consent for Screening Part 2 (SoA, Table 1-3) will be assessed to determine if they meet all eligibility criteria to enter the antenatal treatment phase and to receive treatment with RLYB212 according to the protocol.

Dosing with RLYB212 will be initiated at Visit 3, which must occur no later than GW 16. That is, if the fetal HPA-1 genotype is pending and will not be available by the planned baseline visit at GW 16, the subject will be dosed with the initial dose of RLYB212 pending the result (of the

replicate tests), provided all other Screening Part 2 criteria are met. A second dose of RLYB212 will not be given if definitive fetal HPA-1a results are not available by Visit 4 (~GW 20). If the fetal HPA-1a genotype data become available after the initial dose of RLYB212 is given and the fetus is HPA-1b/b, ie, not at risk for HPA-1a alloimmunization, the participant will be discontinued from treatment, followed at a safety visit 4 weeks later and then discharged from the study to receive normal prenatal care and follow-up by their physician. In this case, the participant would have been determined not to be at risk for development of HPA-1a alloimmunization and FNAIT.

The study has been designed with multiple sequential cohorts (Section 1.2.1). Therefore, if a participant has been determined to be eligible for RLYB212, but the cohort has been fully enrolled, the participant will be requested to consent for the non-interventional (observational) sub-study.

Eligibility for the non-interventional (observational) sub-study is presented in Table 1-1.

8.2 Pharmacokinetic Assessments

Serum samples will be collected for measurement of concentrations of study drug as specified in the SoA (Section 1.3).

The timing of sampling may be altered during the study based on emergent data (eg, to obtain data closer to the time of peak serum concentrations) to ensure appropriate monitoring.

A cord blood sample will be collected at parturition to inform neonate exposure to RLYB212.

8.3 Assessment of Pregnancy and Neonatal Outcomes

8.3.1 Obstetric/Fetal Doppler Ultrasound

Obstetric/fetal doppler ultrasound monitoring will be performed as outlined in the SoA (Section 1.3) and at regular intervals during the study and consistent with local obstetric standard of care, to detect any evidence of placental structural abnormality, organ pathology, hemorrhage, or delayed growth in the fetus, or any doppler velocimetry effects indicative of placental insufficiency or fetal anemia. These assessments will be performed in the second trimester and in the third trimester taking into consideration applicable guidelines, ie, International Society of Ultrasound in Obstetrics & Gynecology (ISUOG) [18-21], local guidelines and other resources (eg, ACOG, CADTH Health Technology Review). Suggested obstetric/fetal doppler ultrasound assessments from these resources include biometry, eg, head circumference, femur length, fetal anatomy (reported as normal OR abnormal [with details] OR not seen, with explanation), placenta, eg, position; fetal cardiac activity, hemorrhage in pregnancy, fetal growth abnormalities, eg, small-for-gestational age/fetal growth restriction, large-for-gestational age/macrosomia; assessment of amniotic fluid volume. Planned doppler assessments include velocimetry of the fetal middle cerebral artery, umbilical and uterine arteries as well as fetal venous waveforms.

Because many of these measurements (particularly doppler vascular measurements) can be done by different techniques (ultrasound modality, transvaginal vs transabdominal, proximal vs distal

umbilical artery), and because multiple indices exist to assess some of the raw ultrasound findings, no attempt will be made to standardize techniques in this small study, so long as all investigators adhere to recommended practices.

8.3.2 Visit Assessments for Pregnancy Outcomes that are Live Births

For pregnancies that result in a live birth, the assessment of alloimmunization at 10 weeks postpartum will include:

- A blood sample collected for the assessment of maternal alloimmunization (refer to the study Laboratory Manual for details), as determined by the presence of detectable anti-HPA-1a alloantibodies.

Other assessments at delivery/parturition will include:

- Pregnancy outcome (Section 3): full term birth, premature birth, mode of delivery, including the date.

- A cord blood sample will be collected at delivery to assess for the possible occurrence of neonatal thrombocytopenia and severe neonatal thrombocytopenia, as determined by a cord blood platelet count $< 150 \times 10^9$/L and $< 50 \times 10^9$/L, respectively.

In the event of neonatal thrombocytopenia, the management and treatment of the event will be recorded in the CRF.

8.3.3 Vital Assessments for Pregnancy Outcomes that are Not Live Births

For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [either spontaneous or elective] or stillbirth). This will include:

- A blood sample collected for the assessment of maternal alloimmunization (refer to the study Laboratory Manual for details), as determined by the presence of detectable anti-HPA-1a antibodies.

Other assessments at the pregnancy-terminating event will include:

- Pregnancy outcome: spontaneous abortion, elective abortion, stillbirth, including the date.

8.3.4 Neonatal/Infant Assessments

8.3.4.1 Parturition

- Collect Appearance, Pulse, Grimace, Activity, and Respiration(APGAR) scores at birth, gender, weight, head circumference, and length.

- Collect cord blood sample for:
  - Platelet count
  - Hematocrit, hemoglobin, reticulocytes, total bilirubin
  - Concentration of RLYB212
- Record any AEs, eg, fetal/neonatal bleeding (Section 10.3.3) [17]
- Record any concomitant therapy.

8.3.4.2 4-6 Weeks Postpartum Visit

- Weight, head circumference, and length
- General health and overall status (absolute values and percentiles)
- Infant behavior
- Record any AEs
- Record any concomitant therapy.

8.3.4.3 12 Months Postpartum Visit

- Weight, head circumference, and height
- General health and overall status (absolute values and percentiles)
- Infant behavior
- Record any AEs
- Record any concomitant therapy
- Assess using a formal, reliable, screening tool that measures sensory, motor, and perception developmental domains against published norms, eg, Ages & Stages Questionnaires®, Third Edition (ASQ®-3).

8.4 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Section 1.3).

8.4.1 Physical Examinations

A complete physical examination will include the following observations/measurements: height, weight, general appearance, skin, head, eyes, ears, nose, and throat, lymph nodes, heart, lungs,

abdomen, extremities/joints, neurological, and mental status. Any significant abnormalities observed at screening will be recorded as medical history.

A brief physical and obstetric examination (heart, lungs, abdomen) will also be performed throughout pregnancy and the postpartum period per the SoA (Section 1.3).

8.4.2 Vital Signs

Systolic and diastolic blood pressures (mmHg), heart rate (beats/minute), and temperature (°C) will be recorded in supine position. Any clinically significant abnormal findings will be recorded as AEs except events identified at the Screening Part 2 visit.

8.4.3 Electrocardiograms

Prior to dosing, a 12-lead ECG will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. A review by a cardiologist of the resultant cardiogram is not required prior to dosing.

8.4.4 Clinical Laboratory Assessments

Maternal clinical laboratory assessments will be obtained during the study as summarized in Section 10.2 (Appendix 2).

The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents.

All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.

If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified, and the Sponsor notified.

All protocol-required laboratory assessments as defined in Section 10.2 (Appendix 2) must be conducted in accordance with the laboratory manual and the SoA (Section 1.3).

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the CRF.

8.5 Adverse Events and Serious Adverse Events

AEs will be spontaneously reported by the participant in response to general questions of how they are feeling. Specific new AEs will not be solicited, although follow-up questions about prior AEs should be asked if not volunteered spontaneously by the participant.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or study procedures, or that caused the participant to discontinue the study or study intervention (Section 10.3 [Appendix 3]).

The definitions of pregnancy-related and fetal AEs can be found in Section 10.3 (Appendix 3) [17].

8.5.1 Time Period and Frequency for Collecting AE and SAE Information

All SAEs will be collected from the Screening Part 2 visit until the end of study visit (10 weeks postpartum for the maternal participant, and 4-6 weeks and 12 months of age for the neonate/infant) at the time points specified in the SoA (Section 1.3).

Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the CRF, not the AE section.

All SAEs will be recorded and reported to the Sponsor or designee immediately, and under no circumstance should this exceed 24 hours, as indicated in Section 10.3 (Appendix 3). The investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE after conclusion of study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the Sponsor.

8.5.2 Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs, and the procedures for completing and transmitting SAE reports, are provided in Section 10.3 (Appendix 3).

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.5.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant or fetus/neonate at subsequent visits/contacts. All SAEs for the participant, fetus, or neonate/infant will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Section 10.3 (Appendix 3).

8.5.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

For all studies except those utilizing medical devices, investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and Sponsor policy, and forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

8.5.5 Adverse Events of Special Interest

There are no AEs of special interest specified in this protocol. Fetal and neonatal outcomes, many of which are AEs, are the focus of this study, as outlined in Section 8.3.

8.6 Measurement of Anti-HPA-1a Alloantibodies

Serum samples will be tested by the Sponsor or Sponsor's designee to assess for the presence of anti-HPA-1a alloantibodies.

An initial anti-HPA-1a alloantibody test will be performed at screening to establish the absence of detectable alloantibodies (ie, lack of existing HPA-1a alloimmunization). An assessment of alloimmunization at the Week 10 postpartum timepoint will inform on the occurrence of HPA-1a alloimmunization in the pregnancy.

The selection of the 10-week postpartum point provides sufficient time since parturition for any potential alloantibody response to fetal HPA-1a exposure during the birth, while also minimizing interference from residual RLYB212 levels in the samples.

Measurement of anti-HPA-1a alloantibody will use the same laboratory test at both the baseline and the Week 10 postpartum time point (Section 1.3).

8.7 Immunogenicity Assessments

Development of ADAs against RLYB212 will be evaluated in serum samples collected from all participants according to the SoA (Section 1.3). To ensure a meaningful interpretation of antibody data, all collected ADA samples will have concurrently RLYB212 serum concentration samples. Serum samples should also be collected at the final visit from participants who

discontinue study intervention or are withdrawn from the study. These samples may be tested by the Sponsor or Sponsor's designee.

The detection and characterization of ADAs against RLYB212 will be performed using a validated assay method by or under the supervision of the Sponsor. The titer of confirmed positive samples will be reported as well as the presence of neutralizing antibodies. Other analyses may be performed to verify the stability of antibodies to RLYB212, and/or further characterize the immunogenicity of RLYB212.

If positive ADA is detected during the study, the PK profiles from participants who were ADA negative and positive will be compared to assess the impact of ADAs on RLYB212 PK.

8.8 Efficacy Assessments

There are no formal efficacy objectives in this study.

8.9 Non-interventional (Observational) Sub-study Assessments

8.9.1 Visit Assessments for Pregnancy Outcomes that are Live Births

For pregnancies that result in a live birth, the assessment of alloimmunization will be at 10 weeks postpartum as defined in Section 8.6.

- A blood sample will be collected for the assessment of maternal alloimmunization (refer to the study Laboratory Manual for details), as determined by the presence of detectable anti-HPA-1a antibodies.
- Pregnancy outcome: full term birth, premature birth, mode of delivery, including the date (based on medical records).
- Occurrence of neonatal thrombocytopenia and severe neonatal thrombocytopenia, as determined by a platelet count $< 150 \times 10^9$/L and $< 50 \times 10^9$/L, respectively, where data are available (based on medical records).
- Interventions used: eg, intravenous immune globulin (IVIG), steroids, fetal platelet transfusions, newborn platelet transfusions.

8.9.2 Visit Assessments for Pregnancy Outcomes that are Not Live Births

For pregnancies that do not result in a live birth, the assessment of alloimmunization will be at 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

- A blood sample will be collected for the assessment of maternal alloimmunization (refer to the study Laboratory Manual for details), as determined by the presence of detectable anti-HPA-1a antibodies.

- Pregnancy outcome: spontaneous abortion, elective abortion, stillbirth, including the date (based on medical records)

- Interventions used: eg, IVIG, steroids

8.10 Independent Data Monitoring Committee

A DMC will monitor safety outcomes and study conduct and will provide recommendations for consideration by the Sponsor regarding the continuation of the study, dosing of further participants in the study or stopping the study for all participants or subgroups of participants all along the study.

Additional details are provided in Section 10.1.5 (Appendix 1).

9 STATISTICAL CONSIDERATIONS

The statistical analysis plan (SAP) will be finalized prior to database lock and will include a technical and detailed description of the statistical analyses described in this section. This section (Section 9.1 through Section 9.5, inclusive) is a summary of the planned statistical analyses of primary and key secondary endpoints with this interventional study.

9.1 Statistical Hypothesis

This study will be descriptive in nature and no hypothesis testing is proposed.

9.2 Sample Size Determination

The primary objectives of the study are to characterize the safety and PK of RLYB212 in HPA-1b/b pregnant women at risk of HPA-1a alloimmunization. Empiric PK data from this study will be used to supplement the parameters for the current clinical pharmacology model, including the impact of target-mediated drug disposition, if necessary. The PK characterization of RLYB212 in this study is intended to be exploratory, rather than to meet a pre-specified statistical objective. The proposed sample size of 8 is consistent with other studies reported in literature intended to characterize the PK of monoclonal antibodies in an exploratory manner and has been determined to be adequate from a PK perspective based on the following:

- *Low to moderate variability observed in RLYB212 PK*: In Study IPA2001 (Section 2.2.2.2), after a single SC dose of 0.21 mg RLYB212 (n = 6), the CV% for RLYB212 exposure in HPA-1b/b volunteers was 32.2% for C_{max} and 26% for AUC_{last}. After repeat SC dosing in Study IPA2001 (0.29 mg of RLYB212 or placebo on Day 1 followed by 0.1 mg on Days 15, 29, 43, 57, and 71; n = 6), observed CV% were reduced to 18.6% for C_{max} and 18% for AUC_{tau}, respectively, on Day 71. In Study IPA2003 (Section 2.2.2.2), in HPA-1b/b volunteers who received an HPA-1a positive platelet challenge after single SC doses of 0.09 mg (n = 4) or 0.29 mg (n = 5) RLYB212, the CV% for RLYB212 exposure ranged from 16.5 and 27.2% for C_{max} and from 16 and 24% for AUC_{last}, respectively. Given the low inter-individual variability in RLYB212 PK observed across 4 distinct dose regimens in 21 HPA-1b/b volunteers at dose levels relevant to those proposed in Study IPA2202, including in volunteers receiving antigen-positive platelet challenge, the proposed sample size of n = 8 is adequate for the stated purpose of an exploratory characterization of RLYB212 PK in HPA-1b/b pregnant women at risk of HPA-1a alloimmunization.

- *Antibody PK in pregnancy*: While physiological changes during pregnancy such as increases in body weight and blood volume are well characterized, published data on maternal exposure to therapeutic mAbs throughout the second and third trimesters are available for only a limited number of mAbs to date. [22, 23] While the effect of pregnancy on exposure parameters is mAb-specific, the variability in the reported PK parameters of individual mAbs is modest through all three trimesters of pregnancy.

- *Conservative dose selection approach*: The modeling and simulation analyses that supported the RLYB212 dose selection for this study incorporated conservative assumptions that minimize the risk of maternal serum RLYB212 concentrations exceeding the ~10 ng/mL

(0.067 nM) target upper boundary concentration (Section 2.1), which is believed to be well below the threshold that could cause harm to the developing fetus. A larger sample size is, therefore, not expected to be informative for the purpose of characterizing PK excursions outside of this upper threshold. PK data from Study IPA2202 will be used to update the modeling and simulation analyses in an iterative manner to inform the dose for the planned Phase 3 study.

It is anticipated that approximately 5,000 participants will be screened to identify 8 pregnant women who both meet the study-specific screening test requirements (ie, genotype HPA-1b/b, genotype HLA-DRB3*01:01 positive, anti-HPA-1a antibody negative, and carrying an HPA-1a positive fetus) and who meet all other inclusion/exclusion criteria to be eligible to receive the study intervention (RLYB212). Women in their first or subsequent pregnancy may be included in this study. Replacement of participants is permitted to achieve 8 participants for whom the pregnancy results in a live birth and who are evaluable for PK and safety assessments (Section 7.2).

9.3 Analysis Sets/Populations for Analysis

For purposes of analysis, the following populations are defined:

Population	Description
Safety Analysis Set	All participants in the study who received one or more doses of RLYB212
RLYB212 PK Analysis Set	All participants for whom at least one concentration above LLOQ is available for RLYB212 will be included in the RLYB212-PK data set for analysis.
Alloimmunization Analysis Set	All participants who had at least one screening (Screening Part 1 or 2) or baseline (Visit 2) determination of anti-HPA-1a activity and a subsequent determination of anti-HPA-1a activity postpartum.

HPA = human platelet antigen; LLOQ = lower limit of quantification; PK = pharmacokinetic.

9.4 Statistical Analyses

The SAP will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses including primary and key secondary endpoints.

9.4.1 General Considerations

For continuous variables, descriptive statistics including number of participants, mean, standard deviation, median, minimum, and maximum will be presented. For categorical variables, the number and percentage of participants will be presented.

Data analysis and presentation will be performed by SAS version 9.4 or later.

9.4.2 Primary Endpoints

The PK profile of RLYB212 during pregnancy will be modelled via a compartmental population PK model with 3 primary parameters, eg, volume of distribution (Vd), clearance (CL), absorption rate constant (k_a) and derived parameters: C_{max}, time to maximum serum

concentration (t_{max}), area under the curve (AUC_{inf}), terminal half-life ($t_{½}$), and elimination rate constant, and a secondary model will provide the following parameters, eg:

PK Parameter	Definition
$AUC_{0\text{-}inf}$	area under the RLYB212 concentration-time curve from time zero extrapolated to infinity
AUC_{tau}	area under the RLYB212 concentration-time curve over the dosing interval
C_{max}	maximum RLYB212 concentration
t_{max}	time to maximum RLYB212 concentration
CL/F	apparent clearance
$t_{½}$	half-life

Safety assessment (Section 9.4.3) comprises the second primary endpoint. No formal pre-specified analysis is specified in the SAP.

9.4.3 Secondary Endpoints

RLYB212 exposure in the neonate will be evaluated by cord blood concentration of RLYB212, which will be a single measurement, and neonatal thrombocytopenia will likewise be evaluated by cord blood platelets.

Other aspects of safety of RLYB212 exposure in the neonate will be evaluated during the postpartum follow-up period in terms of type and seriousness and incidence of AEs, physical examination findings including APGAR scores, vital signs and cord blood levels for platelets, hematocrit, hemoglobin, reticulocytes, total bilirubin, and any additional safety testing done on the neonate on an ad hoc basis.

RLYB212 immunogenicity data and the presence of ADA, and whether there is a neutralizing response, will be determined.

Pregnancy and neonatal outcomes following antenatal RLYB212 administration will include:

- Number of spontaneous abortions, defined as non-deliberate fetal death which occurs prior to GW 19.
- Number of elective abortions, defined as deliberate termination of pregnancy at any time in gestation.
- Number of stillbirths, defined as non-deliberate fetal death anytime in gestation on or after GW 19.
- Number of premature births, defined as live birth prior to GW 37.
- Number of full term births (≥ GW 37).
- Overall health and development of infants at 4-6 weeks and 12 months of age.

- Neonatal thrombocytopenia and severe neonatal thrombocytopenia, as defined by a platelet count < 150×10^9/L and < 50×10^9/L, respectively, collected as a part of the cord blood sample.

Maternal alloimmunization to HPA-1a will be determined by a measurement of maternal circulating anti-HPA antibodies at 10 weeks postpartum or 10 weeks from the date of the pregnancy-terminating event (ie, abortion [spontaneous/elective] or stillbirth).

Where possible, data will be summarized using descriptive statistics and listed.

9.4.4 Tertiary/Exploratory Endpoints

Not applicable.

9.4.5 Other Safety Analyses

All routine safety analyses will be performed on the Safety Analysis Set. Data will be presented separately for maternal participants and neonates/infants.

Safety will be evaluated by presenting summaries of AEs and SAEs, maternal clinical laboratory evaluations (hematology, serum chemistry, coagulation, and urinalysis [Section 10.2 (Appendix 2)]), physical examination, ECGs, vital signs, and RLYB212 immunogenicity data (ADA and neutralizing antibody response). Safety variables will be tabulated for the safety population. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) supplemented with additional information for pregnancy and parturition-related AEs from Spencer et al [17].

A TEAE is defined as an AE that occurs after study treatment is first administered through the end of the follow-up period. The incidence of TEAEs will be presented by system organ class (SOC) and preferred term, by relationship to study drug, and severity. In addition, the incidence of serious TEAEs will be presented by system organ class (SOC) and preferred term (PT), including obstetric TEAEs as outlined in Section 10.3 (Appendix 3), which will be categorized via a dictionary extension specific for these events. Descriptive statistics for clinical laboratory test results, ECGs, and vital signs, including changes from baseline, will be presented by time point. Incidences of potentially clinically significant clinical laboratory results, ECGs, and vital signs, as defined in the SAP, will also be summarized by time point. Frequency and percentages of physical exam findings will be summarized.

Regular obstetric/fetal doppler ultrasounds as scheduled by the protocol will be included in the safety evaluation.

9.5 Interim Analyses

No formal interim analyses will be conducted. Preliminary data may be used for summary purposes and for communication with health authorities.

9.6 Non-interventional (Observational) Sub-study

This sub-study is intended to be descriptive in nature; no hypothesis will be tested in this sub-study. Demographic and baseline characteristics will be summarized. Discrete variables will be summarized using numbers and percentages; continuous variables will be summarized using means and SD or medians and IQRs, as appropriate. Administrative tabulated summaries of study variables will be generated periodically. Data obtained from the non-interventional (observational) sub-study will be pooled with those acquired from study IPA2002 (NCT05345561) for analysis of natural history variables of the higher risk FNAIT population.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
- Applicable ICH Good Clinical Practice (GCP) Guidelines.
- Applicable laws and regulations.
- The protocol, protocol amendments, informed consent form (ICF), investigational brochure, and other relevant documents (eg, advertisements) must be submitted to an independent review board (IRB)/independent ethics committee (IEC) by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
- Notifying the IRB/IEC of serious adverse events (SAEs) or other significant safety findings as required by IRB/IEC procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of Code of Federal Regulations Title 21 (21 CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2 Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3 Informed Consent Process

The investigator or his/her representative will explain the nature of the study to the participant and answer all questions regarding the study.

Participants must be informed that their participation is voluntary and will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants will complete an ICF for the initial screening (Screening Part 1) in the study prior to assessing eligibility. Participants who are then determined to be eligible for study treatment at the subsequent screening (Screening Part 2) are required to sign a new ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of all ICFs (plus any ICFs updated while the participant is enrolled in the study) must be provided to the participant.

10.1.4 Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that their personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.

The participant must be informed that their medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committee Structure

In this study, an independent data monitoring committee (DMC) will monitor safety outcomes and study conduct and will provide recommendations for consideration by the Sponsor regarding continuation of the study, dosing of further participants in the study, or stopping the study for all participants or subgroups of participants in the study. Safety data will include, but not be limited to, those outcomes listed as primary and secondary endpoints.

The DMC is a multidisciplinary group composed of experts, physicians experienced in the treatment of the disease under investigation, FNAIT.

The Sponsor will propose a detailed mandate and review this with the DMC, from the outset (a detailed DMC charter and operating rules will be prepared and signed by DMC members before study start).

The DMC will review study data at regular intervals as described in the DMC charter. These sessions could be regular sessions or ad-hoc sessions. Ad-hoc sessions can be requested by the Sponsor or the DMC as needed and at any time during the study.

10.1.6 Dissemination of Clinical Study Data

The Sponsor will register and/or disclose the existence of and the clinical study data as required by local laws and regulations.

10.1.7 Data Quality Assurance

All participant data relating to the study will be recorded on printed or electronic CRFs unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy (eg, risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

The Sponsor or designee is responsible for the data management of this study including quality checking of the data.

The Sponsor assumes accountability for actions delegated to other individuals (eg, CROs).

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents, that the safety and rights of participants are being protected, and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the

retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

10.1.8 Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Definition of what constitutes source data can be found in the Location of Source Data form provided to the site prior to the start of the study.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.9 Study and Site Start and Closure

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of participants by the investigator.
- Discontinuation of further study intervention development.

10.1.10 Publication Policy

The Sponsor and the investigator(s) will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of the sites' study results following publication of the multicenter study results in their entirety. The Sponsor expects that a coordinating investigator will be designated to oversee publication of the multicenter study results. Authorship will be determined in line with International Committee of Medical Journal Editors authorship requirements.

If the investigator is authorized to publish individual site data, the investigator agrees to submit all manuscripts, publications, or abstracts to the Sponsor before submission. This approach allows the Sponsor to protect proprietary information.

10.2 Appendix 2: Clinical Laboratory Tests

The tests detailed in Table 10-1 will be performed by central laboratories and local laboratories.

Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol.

Investigators must document their review of each laboratory safety report.

Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 10-1 Protocol-required Clinical Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet count RBC count Hemoglobin Hematocrit	RBC indices: • MCV • MCH • MCHC • % reticulocytes		Platelet count WBC count with differential: • Neutrophils • Lymphocytes • Monocytes • Eosinophils • Basophils
Clinical Chemistry	Urea Albumin Creatinine Glucose (nonfasting)	Potassium Sodium Calcium C-reactive protein	 ALT/SGPT AST/SGOT GGT	Total bilirubin (and direct and indirect bilirubin[a]) Total protein Alkaline phosphatase
Coagulation	Prothrombin time International normalized ratio Activated partial thromboplastin time			
Viral Serology	Hepatitis C virus antibody; Hepatitis B surface antigen (HBsAg); HIV antibody			
Routine Urinalysis	Specific gravity Dipstick for glucose, pH, nitrate, occult blood, ketones, bilirubin, urobilinogen, nitrite, and leukocyte esterase Microscopic examination (if blood or protein is abnormal)			
FNAIT Testing [δ]	FNAIT laboratory screening tests for maternal HPA-1a genotype, maternal HLA-DRB3*01:01 genotype, and maternal HPA-1 alloantibody[b], fetal HPA-1a genotype/cffDNA			
RLYB212 Specific [δ]	Immunogenicity data (ADA response and presence of neutralizing antibody) PK			

ADA = anti-drug antibody; ALT = alanine aminotransferase; AST = aspartate aminotransferase; cffDNA = cell-free DNA; FNAIT = fetal and neonatal alloimmune thrombocytopenia; GGT = gamma glutamyl transferase; HBV = hepatitis B virus; HCV = hepatitis C virus; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PK = pharmacokinetics; RBC = red blood cell; SGOT = serum glutamic-oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase; WBC = white blood cell.

[a] Direct and indirect bilirubin will only be assessed if total bilirubin is elevated.

[b] If a positive test result is observed for anti-HPA-1a maternal alloantibody test, the results should be discussed with the Sponsor (Section 8.4.4).

[δ] FNAIT testing and RLYB212 specific tests are performed in central laboratories; all other tests are performed locally.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1 Definition of Adverse Events

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • For efficacy studies, “lack of efficacy” or “failure of expected pharmacological action” per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant’s condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant’s condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or conditions(s) present or detected at the start of the study that do not worsen.

10.3.2 Definition of Serious Adverse Events

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
Results in death
Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
Results in persistent disability/incapacity The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
Is a congenital anomaly/birth defect
Other situations: Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive intervention in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3 Definitions of Pregnancy- and Parturition-specific Adverse Events

Maternal and Fetal Adverse Events
Maternal AEs: • Haemorrhage in pregnancy • Preterm premature rupture of membranes • Chorioamnionitis • Anaemia of pregnancy • Gestational hypertension • Pre-eclampsia • Eclampsia • Premature labour • Puerperal infection • Postpartum haemorrhage (primary) • Retained placenta or membranes • Amniotic fluid embolism

Fetal AEs:

- Haemorrhage in pregnancy
- Preterm premature rupture of membranes
- Chorioamnionitis
- Anaemia of pregnancy
- Fetal fluid collection[a]
- Fetal bradycardia: non-labour[a]
- Fetal tachyarrhythmia[a]
- Cardiac function abnormalities[a]
- Fetal brain scan abnormal[a]
- Fetal gastrointestinal tract imaging abnormal[a]
- Fetal musculoskeletal imaging abnormal[a]
- Fetal renal imaging abnormal[a]
- Fetal movement disorders[a]
- Fetal neoplasm
- Fetal structural abnormalities: not otherwise classified[a]
- Abnormal fetal growth[a]
- Fetal intraoperative injury[a]
- Procedure haemorrhage[a]
- Post-procedural haemorrhage[a]

[a] Added to the list of terms in the MedDRA.

Source: [17].

Maternal and Fetal AE Definitions and Severity Grading Criteria

Guidance on the use of these severity grading criteria:

- If an adverse event fulfils the criteria for more than one grade of severity, the highest applicable grade should be used.
- Death resulting from any of the adverse events is classified as grade 5.
- A semicolon indicates 'or' within the description of a grade.
- Maternal procedural complications, such as pain and infection, should be identified by the appropriate MedDRA preferred term and graded according to CTCAE criteria.
- Maternal thromboembolic events during pregnancy and the puerperium should be identified by the appropriate MedDRA preferred term ('venous thrombosis in pregnancy', 'postpartum venous thrombosis', or 'obstetrical pulmonary embolism') and graded according to the CTCAE criteria 'thromboembolic event'.

Source: [17].

10.3.4 Recording and Follow-up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to the pharmacovigilance CRO in lieu of completion of the AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by the regulatory authorities. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort, and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult the IB and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to the pharmacovigilance CRO. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to** the pharmacovigilance CRO**.**
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the pharmacovigilance CRO to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- After the initial AE/SAE report, the investigator is required to proactively follow each participant or fetus/neonate at subsequent visits/contacts.
- All SAEs reported for the participant, fetus, or neonate/infant will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the pharmacovigilance CRO with a copy of any postmortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to the pharmacovigilance CRO within 24 hours of receipt of the information.

10.3.5 Reporting of SAEs

SAE Reporting to the Pharmacovigilance CRO via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE to the pharmacovigilance CRO will be the electronic data collection tool. • If the electronic system is unavailable, then the site will use the paper SAE data collection tool to report the event within 24 hours. • The site will enter the SAE data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form or to the pharmacovigilance CRO by telephone.

10.4 Appendix 4: Abbreviations

Abbreviation	Definition for Term
21 CFR	Code of Federal Regulations Title 21
ADA	anti-drug antibody
AE	adverse event
ALT	alanine aminotransferase
APGAR	Appearance, Pulse, Grimace, Activity, and Respiration
AST	aspartate aminotransferase
AUC	area under the (RLYB212) concentration versus time curve
$AUC_{0\text{-}inf}$	area under the concentration-time curve from time zero extrapolated to infinity
cffDNA	cell-free fetal DNA
CIOMS	Council for International Organizations of Medical Sciences
CL	clearance
CL/F	apparent clearance
C_{max}	maximum serum concentration
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CRO	Contract Research Organization
CRP	C-reactive protein
CTCAE	common terminology criteria for adverse events
DMC	Data Monitoring Committee
ECG	electrocardiogram
EOS	end of study
FNAIT	fetal and neonatal alloimmune thrombocytopenia
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
GLP	Good Laboratory Practice
GW	Gestational Week
HBV	hepatitis B virus
HCV	hepatitis C virus
HDFN	hemolytic disease of the fetus and newborn
HIPAA	Health Insurance Portability and Accountability Act
HLA	human leukocyte antigen
HPA	human platelet antigen
HPLC	high performance liquid chromatography
ICF	informed consent form

Abbreviation	Definition for Term
ICH	intracranial hemorrhage
ICTMG	International Collaboration for Transfusion Medicine Guidelines
IEC	Independent Ethics Committee
Ig	immunoglobulin
IRB	Institutional Review Board
ISUOG	International Society of Ultrasound in Obstetrics & Gynecology
ITP	immune thrombocytopenia
IVIG	intravenous immunoglobulin
k_a	absorption rate constant
LDH	lactate dehydrogenase
LLOQ	lower limit of quantification
mAb	monoclonal antibody
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PoM/PoC	proof of mechanism/proof of concept
PT	preferred term
Q4W	every 4 weeks
RBC	red blood cell
RhD	Rhesus D
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous(ly)
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SoA	schedule of activities
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
$t_{½}$	half-life
TCR	tissue cross reactivity
TEAE	treatment-emergent adverse event

Abbreviation	Definition for Term
TK	toxicokinetic(s)
t_{max}	time to maximum (RLYB212) concentration
ULN	upper limit of normal
Vd	volume of distribution
WBC	white blood cell
WT	wild-type

10.5 Appendix 5: Protocol Amendment History

Version 4.0 (14 October 2024) superseding Version 3.0 (25 September 2024)

Rationale:

The SoA was updated to indicate that samples for hematology, chemistry, coagulation, and CRP assessments will be collected at Screening Part 2, Visit 2.

Version 3.0 (25 September 2024) superseding Version 2.0 (04 September 2024)

Rationale:

The following changes were made based on feedback received from the European Medicines Agency (EMA):

- Follow-up of the infant to 12 months of age; modifications were incorporated in the Synopsis, Schema/SoA, Objectives/Endpoints, End of Study Definition and Study Assessments and Procedures.

In addition, corrected EU CT number (removal of submission number).

Version 2.0 (04 September 2024) superseding Version 1.1 (23 August 2024).

Rationale:

The following changes were made based on feedback received from the European Medicines Agency (EMA):

- Included clarification that the occurrence of neonatal thrombocytopenia following antenatal RLYB212 administration will be evaluated based on the umbilical cord sample taken at parturition.
- Updated Exclusion Criteria at Screening Part 2:
    - Revised exclusion criterion 4 to: “Any participant with a history of past or current intravenous immunoglobulin (IVIG) use for an underlying disease (eg, autoimmune disease)”
    - Replaced exclusion criterion 7 with new criterion: “Participants with an in vitro fertilized pregnancy or serving as pregnancy surrogates”
- Clarified that broad spectrum immunosuppressant agents and IVIG are not allowed as concomitant medications with RLYB212 administration. If such treatments are required, RLYB212 will be discontinued but the participant will continue to be followed in the study.

- Replaced statement that vaccinations are not allowed within 30 days of screening until 30 days after the last dose of study drug, with "Routine vaccines, given at recommended times during or immediately after pregnancy, will be allowed in the study."

- Clarified that up to 4 participants may be replaced if *assigned to study drug* but did not receive the study drug.

- Removed statements that samples collected as part of the screening and monitoring procedures may be retained for the purposes of analytical method validation, or for optional exploratory research.

In addition:

- The description of the sub-study was changed to "non-interventional (observational)".

- For clarity, reference to "live birth" was revised to "full term birth", where applicable.

- Updated the window for the last dose of RLYB212 to be administered from "within 72 hours of parturition" to "within 24 hours (±24 hours) of parturition".

- Included additional explanatory notes to the SoAs; removed "drug testing" from the SoA (Table 1-3); and removed "neonatal platelet count" from the SoA (Table 1-3), as it is addressed by the "cord blood sample" assessment.

- Paragraph on Lifestyle Considerations was simplified.

- Removed references to a Study Reference Manual (not applicable to the study).

- Removed appendix on liver safety (as this was addressed in Phase 1 studies).

Version 1.1 (23 August 2024) superseding Version 1.0 (11 March 2024).

Rationale:

The following changes were made based on feedback received from the United Kingdom Medicines and Healthcare product Regulatory Agency (MHRA), in addition to including the NCT number on the title page:

- Included a description in the protocol of the composition of the independent data monitoring committee that will be overseeing the safety of study participants.

- Included the requirement that after an initial AE/SAE report, the investigator must proactively follow each participant or fetus/neonate/infant at subsequent visits/contacts. All SAEs for the participant or fetus/neonate/infant will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.

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